BIOLUMINESCENCE OF THE SEA

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BIOLUMINESCENCE OF THE SEA

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Luminescence in the Main Groups of Marine Organisms

Luminescence in marine organisms can be an extracellular or intracellular process. Extracellular luminescence is characterized by the release of a luminous secretion produced by specialized glands (unicellular or multicellular). Aggregations of such glands of different size and form may be differentiated from the remaining tissues of the organism and form luminous organs. These organs are usually surrounded by muscle fibers and are sometimes innervated. The secretion of the luminous slime from these organs occurs under stimulation which contracts the muscle fibers and compresses the the secretion-holding glands or cavities. The luminous slime is ejected through a system of special ducts. /9 /10

Extracellular luminescence has been detected in the polychaetes Chaetopterus (Hasama, 1941), Polycirrus and Odontosyllis; Crustacea (Copepoda, Ostracoda, Mysidacea, Decapoda); Gastropoda (Phyllirrhoe), Lamellibranchia (Pholas); Hemichorda (Balanoglossus minutus and Pthychodera bahamensis) (Harvey, 1952). Fish having extracellular luminescence are also known, for example: Searsia koefoedi, S. schnakenbecki (Nicol, 1958a).

Most luminescent organisms have intracellular luminescence. The simplest example of intracellular luminescence is that of unicellular animals (Flagellata) which is not accompanied by any discharge of secretion. In the case of this kind of luminescence in Metazoa, the light comes from special photogenic cells--often forming simple aggregates in different parts of the body (for example, Ctenophora, Polychaeta of the genera Polynoë and Acholoë). In the Cephalopoda, euphausiids, and fish, the photogenic cells are usually situated in recesses of the body integuments surrounded by transparent tissues that serve as lenses, pigmented layers (reflectors), and different kinds of structures that occasionally cover the photogenic cells (screens). Such differentiated aggregates of photogenic cells, usually complicated by additional structures that provide for focussing and reflecting luminous flux from the intima, are called photophores. Luminescent fish and Crustacea are known whose photophores can rotate. Photophores are usually well innervated: the operation of the lenses, screens, and diaphragms is controlled by the nervous system.

Besides the two kinds of luminescence mentioned, a third type, chiefly encountered in Cephalopoda and fish, is known. The luminescence of animals of this type is generated by symbiotic luminous bacteria. The luminous bacteria usually inhabit special cavities surrounded by pigmented and transparent tissues. In structure, such luminous organs resemble photophores, but differ in that the role of the photogenic cells is played by luminous bacteria.

Detailed information on the luminescence of many organisms, from bacteria to fish, and on the structure of their light organs can be found in N. Harvey's classic monograph Bioluminescence (1952). Here we shall only examine the luminescence of those organisms which contribute more or less substantially to the bioluminescence of the sea. Major attention will be focused on data obtained in recent years.

Protozoa--as has already been noted, the luminescence of the most simply organized bioluminescents--the unicellular Radiolaria and Flagellata--is intracellular. /11

Observation of the luminescence of Noctiluca miliaris by the unaided eye creates the impression that it arises diffusely in the cell. However, microscopic investigations (Harvey, 1917a; Eckert, 1965a,b; Eckert, Reynolds, Chaffee, 1965) have shown that in Noctiluca, the flashes are generated in many tiny sources--organelles-having a diameter of about 0.5 micron, located exclusively along the cell periphery (Eckert, Reynolds, 1967). With the aid of image converters, it was possible to record the luminescence of individual organelles--"microflashes" (fig. 1). Records of microflashes had the same form as those of macroflashes (luminescence of the entire cell) and the same time characteristics, with the exception of the decay time. A macroflash of Noctiluca is the sum of many microflashes, each of which occurs in a separate organelle--the elementary functional unit of luminescence. The number of such microsources in a cell having 500-micron diameter is, on the average, equal to $1.6 - 3.6 \cdot 10^4$, and depends on the physiological state of the cell. Unfortunately, as yet nothing is known about the structure of these organelles. It has been established, however, that they do not have anything in common with such organelles, highly visible to light microscopy, as mitochondria and Golgi bodies (Eckert, 1965a). /12

In the cells of another flagellate--Gonyaulax polyedra--luminous particles have also been detected. A group of investigators (De Sa, Hastings, Vetter, 1963; Hastings, Vergin, De Sa, 1966) by means of special techniques succeeded in isolating from G. polyedra cells crystalline granules called scintillons, which when stimulated (change in pH of the medium) produced flashes indistinguishable from those of the G. polyedra. The size of the scintillons, which are rhombohedral in form, range from 0.1 to 0.6 microns in length.

Luminous crystalline particles, close to G. polyedra scintillons in size and form, have been isolated from Noctiluca miliaris cytoplasm (Hastings, Vergin, De Sa, 1966). The luminescence of Noctiluca scintillons is easily evoked by a rapid drop in the pH (to 5.7). They speculate that the luminescing crystals separated from Noctiluca are analogous to the microsources detected by R. Eckert in the intact cell.

Electron microscope photographs of two sectors of G. polyedra cytoplasm with scintillons are shown in Figure 2. It is interesting that many particles which apparently possess crystal properties and resemble G. polyedra scintillons in form and size, are detected by the electron microscope in the cytoplasm of nonluminous Flagellata like Prorocentrum micans and Gymnodinium nelsoni (Sweeney, Bouck, 1966). It is possible that structures analogous to G. polyedra scintillons and Noctiluca microsources are present in the cytoplasm of all Flagellata, but that the particles are only able to luminesce under certain conditions.

Coelenterata and Ctenophora--the bioluminescents of these groups /13
have simple photogenic organs, usually aggregates of either specialized luminescent cells (intracellular luminescence) or of glands that release a luminescent secretion.

In the Hydromedusae, luminescence is observed along the rim of the bell, at the tentacle base (Aequorea, Mitrocoma), or along the radial canals (Phialidium) (fig. 3). Microscopic investigations of /14
the luminous parts of the hydromeduse body have shown that they are filled with yellow cells which constitute the photogenic tissue. During cytolysis of these cells, caused by the addition of fresh water or saponin, it is possible to see that the source of luminescence is large, disc-shaped granules that fill the cells. The size of the luminescent discs varies. The discs luminesce very brightly for a period of time, after which they fade (Harvey, 1952). The luminescence of Hydromedusae is unquestionably intracellular. The luminescent fluid sometimes observed when Hydromedusae are investigated represents the contents of destroyed photogenic cells.

Among the Scyphozoa, Pelagia noctiluca is best known for its ability to luminesce. According to data obtained by P. Panceri (Harvey, 1952) and U. Dahlgren (1916), P. noctiluca luminescence is extracellular. In the luminescent secretion expelled by the jellyfish, P. Panceri detected stinging cells together with epithelial cells filled with small yellow granules. Possibly, these cells are the source of the luminescence.

Among the Ctenophora, luminescence is observed in granular structures along the radial canals (fig. 4). It is not yet clear whether the luminescence occurs in the photogenic cells themselves or whether they release a luminescent secretion (Nicol, 1960a).

In the opinion of D. V. Naumov (1961), there is a definite relationship between the ability of jellyfish to luminesce and their color. All luminous jellyfish are well pigmented: Aequorea aequorea and Halopsis ocellata are pink, Aeginura grimaldii and Periphylla periphylla are brown, while Atolla wyvillei is reddish brown, and so on. Consequently, D. V. Naumov believes that colored deep-water jellyfish (Meator rubata, Calycopsis nematophora, Halicreas minimum, Botrinema brucei, Crossota brunnea, Pantachogon haeckeli, Aegina citrea) should be luminescent.

Mollusca--Mollusc luminescence is encountered among Gastropoda, Lamellibranchiata, and Cephalopoda; the most luminescents being among the Cephalopoda (in the order Decapoda). Of the 400 species in this order, 126 are luminescent (Berry, 1920). According to data obtained by K. N. Nesis (personal communication), more than 140 luminescent species are estimated to be in the suborder Oegopsida alone.

All three types of luminescence are encountered among the Cephalopoda: extracellular, intracellular, and that due to symbiotic luminous bacteria. The most numerous group of species of Cephalopoda has intracellular luminescence. These molluscs belong to the suborder Oegopsida and have a well developed system of numerous specialized photophores. The photophores may be located in any part of the squid's body--on the head, around the eyes, on the mantle, tentacles, or within the mantle cavity (fig. 5). The number of photophores is usually great and varies from species to species. For example, the deep-water squid Pyroteuthis margaritifera has six photophores around each eye and ten on the ventral surface of the body. Pterygioteuthis giardi has 12 photophores around each eye and seven on the ventral surface of the body; Nematolampas regalis (length 57mm) has 92 photophores; and the Watasenia scintillans (length to 10cm) has more than a thousand small photophores. /15

Squid photophores have different structures and therefore differ with respect to the degree of complexity, but basic to them is the radiating tissues proper, consisting of photogenic cells. The photophores are complicated by the inclusion of additional elements--reflectors, lenses, screens, with which the squid regulate the brightness of the luminescence. For example, the photophores of Liocranchia valdiviae, which are located around the eyes, have a simple structure: they are recesses in the epidermis lined with connective tissue that serves as reflector and filled with a granulated mass of emissive /17

cells (Fig. 6); the tentacle photophores of Watasenia scintillans and Abraliopsis morrisi are aggregates of large photogenic cells surrounded by a connective tissue membrane and a chromatophore screen. The structure of the complicated Calliteuthis reversa photophore is shown in Figure 7. In some cases, the chromatophore screen and photogenic tissues of the photophores are permeated with blood vessels and nerve fibers (Nicol, 1960a).

Luminescent Cephalopoda of the suborder Myopsida are significantly inferior to representatives of the suborder Oegopsida with respect to the number and brightness of the luminous organs. This is apparently due to the fact that almost all luminescent Myopsida do not have independent luminescence; it occurs because of symbiotic luminous bacteria. Only two species are exceptions: Heteroteuthis dispar and Sepiolina nipponensis, which eject a luminescent secretion into the water. The luminous organ of H. dispar is located immediately behind the anus and is surrounded by a small ink sac. It consists of photogenic cells that release a luminescent secretion, transparent structures that act as lenses, a reservoir with two ducts, and muscle tissue, the contraction of which releases the secretion into the mantle cavity. From there, the luminescent secretion is ejected through the funnel (Harvey, 1952).

The organs of luminescence with symbiotic bacteria in Cephalopoda /18
are few in number (usually two) and are almost always located in the mantle cavity near the ink sac. The structure of these organs resembles that of photophores (here again reflectors, lenses, and screens are encountered; only the role of the photogenic cells is played by the luminous bacteria). The organ of luminescence of Sepiola ligulata is a saccular recess of the mantle epithelium that is connected to the mantle cavity by two ducts. In the recess are the reflector and a transparent body that acts as lens, the interior of the organ is occupied by the luminous bacteria. In the mollusc Uroteuthis bartschi, the luminous organ is two opaque fabiform bodies of lemon-yellow color, located along both sides of the ink sac. Each fabiform body contains a lens, reflector, and a black membrane which covers part of the lens and serves as a diaphragm. Inside the fabiform body is glandular tissue permeated with blood vessels and filled with luminous bacteria. Mechanical stimulation of the mollusc produces a brief bright flash of light. Inasmuch as the luminescence of the bacteria is constant, the fading observed apparently is due to screening of the lens by the black membrane. A pure bacterial culture that luminesces with blue light at room temperature has been isolated from U. bartschi (Haneda, 1963). It must be said that to date pure cultures of luminous bacteria have been isolated from very few luminescent Myopsida (from Sepiola rondeletii, Loligo edulis, Rondeletiola minor--see Chapter III). In most cases, the bacteria isolated from the photogenic organs of Myopsida do not luminesce in a pure culture.

To date, it is not clear how or when luminous bacteria get in the luminous organs of the host mollusc. Previously, it was thought that luminous bacteria in molluscs are transmitted from generation to generation via eggs. However, it has since been shown that the anlagen of luminous organs in the embryos do not contain the bacteria and that, consequently, the bacteria are not passed along transvarially. "Infection" with luminous bacteria occurs anew in each generation (Nicol, 1960a). The need to renew the symbiosis with the luminous bacteria each generation is probably the reason why nonluminescent specimens are encountered together with luminescents, often even among individuals of the same species and sex. Thus, only half the individuals of the cuttlefish Sepiola rondeletii checked for luminescence up to the present time luminesced (Nicol, 1960a). Without question, this interesting question requires further study.

Among the Gastropoda and Lamellibranchiata, luminescent species are few. Unlike the Cephalopoda, luminescent Gastropoda and Lamellibranchia have simply constructed luminous organs. Usually, they are aggregates of gland cells secreting luminescent slime. Of the luminescent Gastropoda, the mollusc Phyllirrhoe bucephala is best known. The entire body of this pelagic gastropod of the order Nudibranchia, which is beautiful at night, is covered by brightly luminous spots (fig. 8). Each such spot corresponds to a single photogenic cell or aggregate of them sunk in the epidermis. These rather large cells are filled with a granular secretion that can be ejected. A nerve runs to each photogenic cell, forming a thickening on its lateral wall. /19

Of the subclass Prosobranchia of the Gastropoda, only one luminescent species, Tonna galea, was known until recently (Harvey, 1952). Recently, however, luminescence was found in five more representatives of this subclass, belonging to the genus Planaxix: Planaxix virgatus, P. periscelida, P. longispira, P. lineatus, and P. labiosis (all of these species inhabit the coastal waters of Japan and the Hawaiian Islands). These small marine snails withstand aquarium cultivation very well. The luminous organ of P. labiosis is located in the dorsal part of the mantle and has pale blue coloration. The outer, visible part of this organ consists of many parallel folds of luminescent tissue. After special treatment, this tissue, when illuminated by ultraviolet light, displays a green-blue fluorescence. In all five species, the luminous organ is located in the same part of the mantle. Histological investigations have shown that this organ in Planaxix is a mass of fine, tightly packed photogenic cells. Luminescence in Planaxix is probably intracellular, since no release of luminescent secretion has been detected (Haneda, 1958a).

Among the Lamellibranchiata, only two luminescent species are known. One of these, Pholas dactylus, was known in antiguity, while the luminescence of the second, Rocellaria grandis, was discovered at the end of the 1930's (Haneda, 1939). Pholas dactylus discharges luminescent slime into the current siphon from three different sections: a narrow band of photogenic tissue on the anterior edge of the mantle, two of such narrow bands running along the excurrent siphon, and two cells of irregular shape located in the mantle cavity (fig. 9). All of these luminescent sections are aggregates of elongated gland cells filled with easily distinguished luminescent granules, which are discharged through long ducts from the cells to the excurrent siphon. The aggregates of gland cells are permeated with nerve fibers. /20

The location of the luminescent sections on the body of Rocellaria grandis differs slightly from that described above for Pholas dactylus, but in this case too the luminescent slime is discharged by an aggregate of gland cells (Haneda, 1939).

Crustacea--Luminescent Crustacea occur among Ostracoda, Copepoda, Mysidacea, Amphipoda, Isopoda, Euphausiacea, and Decapoda.

Ostracoda. Of the luminescents of the order Ostracoda, Cypridina hilgendorfii, encountered in abundance along Japan's Pacific Coast, is best known. Dried Cypridina, when moistened, luminesce with the same blue color as living specimens. This property of retaining the ability to luminesce over a long period of time explains why C. hilgendorfii is a favorite subject of bioluminescence investigations. The luminescence of Ostracoda occurs when a secretion, produced by special organs--multicellular glands--is released into the water. The arrangement and structure of these glands has chiefly been studied in the example of Cypridina hilgendorfii. The gland that releases luminescent secretion in C. hilgendorfii is located on the labrum (Doflein, 1906; Yatsu, 1917; Okada, 1927; Takagi, 1936). It includes four types of cells that open through ducts into five protuberances (papillae); one anterior medial, two middle, and two posterior. Between the cells of all four types pass many muscle fibers, contraction of which forces the contents of the cells through the papillae to the outside. Granules of cells of the first and second types dissolve well in water and luminesce when mixed (Harvey, 1952).

Copepoda. Luminescence of the Copepoda, as of the Ostracoda, is extracellular. However, unlike Cypridina, which has only one photogenic organ, Copepoda release the luminescent secretion from many glands scattered over the entire body. There is no single opinion relative to the number and arrangement of the luminescent glands in the different species (Harvey, 1952; David, Conover, 1961; Clarke, /21

Conover et al., 1962). An attempt was made to localize the organs of luminescence using the property of photogenic tissues of some Copepoda to fluoresce in ultraviolet light (Clarke et al, 1962). This fluorescence had been detected as early as 1916 (Harvey, 1916). In ultraviolet light, fluorescence of the luminescent glands of various species of Metridia, Pleuromamma, Lucicutia, Euaugaptilus, and Centraugaptilus is observed. The glands of species of the genera Heterorhabdus, Heterostylites, Hemirhabdus, and Disseta do not fluoresce. In the genus Metridia, localization of the luminescent glands is constant in individuals of one species; but differs from species to species. Thus, M. lucens and M. longa have a similar gland arrangement on the urosome but in M. lucens the glands are found on the second thoracic segment, while in M. longa, they are not. The number and arrangement of glands on the head differ most sharply (fig. 10). On the head of M. lucens, there are always ten glands, in M. longa there are 11-15. The arrangement of M. lucens glands, as determined by G. Clarke et al. (Clarke, Conover, et al., 1962) does not correspond to the description given by C. David and R. Conover (1961). In the opinion of the latter, M. lucens has glands on the third and fourth thoracic segments and on all segments of the urosome. /22

In Lucicutia grandis only one pair of luminescent glands, located along the anterior ventral border of the first thoracic segment, has been detected. In Pleuromamma robusta three fluorescent glands have been located on the dorsal side near the rostrum, two at the level of the mandibles, two on the second thoracic segment, and two glands each on the anal segment and caudal rami. In some individuals of Euaugaptilus spp. and Centraugaptilus spp., large fluorescent glands have been found on the distal segments of the swimming legs and on the setae. No differences were found in the gland arrangement of the different species of these genera. For Heterorhabdus norvegicus it has only been possible to determine general regions of luminescent activity, inasmuch as the glands of this species do not fluoresce under ultraviolet light. Some of the luminescent glands of H. norvegicus are located on the head, on the thoracic and anal segments, and on the caudal rami. There are also glands on most cephalic and thoracic appendages. On the distal parts of the swimming legs, glands were sometimes seen under ordinary illumination.

Very little is known about the structure of the luminous organs of the Copepoda. Histological investigations of the photogenic glands of some Copepoda have been conducted only by G. Clarke and Conover (1962). According to their data, the luminescent glands are ampullae, 65-70 microns long and 20-25 microns wide, lying beneath the hypodermis and narrowing to 5-6 microns at the cuticle (fig. 11). The lateral walls of the ampullae are thin, to 2 microns, at the base the wall thickens to 4 microns. The cytoplasm at the base of the ampulla is granulated and stains with hematoxylin. It contains one or two nuclei.

Within these cells are quite large particles containing the luminescent material. These particles are not the same in different cells: in some they stain well with aniline blue to a blue color, in others to red with azocarmine, so that after staining the first cells were filled with blue (type I), and the other group with red particles (type II). Glands of types I and II usually are side by side and share a common duct in the cuticle. The particles in type I glands are very diverse in form, most frequently spherical or polyhedral, 2-6 microns in size. Within a cell they are usually arranged in groups. These particles are filled with homogeneous or fine-grained contents. Particles in type II glands also vary in form; they are polygonal, rectantular, or pyramidal, with size of about 7-11 microns. They usually display pronounced striation. The particles of both kinds form in the ampulla bases and then move towards the duct. The ampullae are surrounded by muscle fibers, the contraction of which apparently causes the discharge of the particles contained therein into the water. Metridia lucens has type I and II glands whose structure is the same as in M. longa. The same glands are found in Pleuromamma robusta. Particles from the glands dissolve well in sea water; a luminous cloud forms in the water when they are ejected simultaneously.

Mysidacea--the few luminescent representatives of this order have extracellular luminescence. The organ of luminescence has been best studied in the mysid Gnathophausia calcarata and is an aggregate of gland cells on the maxilla. The luminescent secretion is collected in a special reservoir that is connected by a duct to the papilla, which opens to the outside. The release of the secretion into the sea water apparently occurs as the result of the contraction of muscle fibers surrounding the reservoir.

Euphausiacea--Only intracellular luminescence is known among the Euphausiacea. The luminous organs here are well developed photophores located on the optic stalks, the abdomen, and the thorax. The number of photophores in Euphausiacea can vary from three (Stylocheiron) to ten (Meganyctiphanes). The small circular photophores of euphausiids lie in pairs in the base of the thoracic legs, as well as in the base of the first four pairs of swimming legs. The photophores of the optic stalks of the Euphausiacea are large and pear-shaped. They are located inside the eye; the photophores are separated from the optic elements (ommatidia) by a black pigment screen. In the quiescent state (motionless eye) the light from these photophores is reflected down and back.

The photophores of Euphausiacea can be simple or complex. Simple photophores are cup-shaped, within which the photogenic tissues are immersed in a mass of rod-shaped cells. On the top, this cup is covered by a refracting lamellate body. More complex photophores (Meganyctiphanes norvegica) contain lenses, a reflector, and a pigment layer. In the photophores of Nyctiphanes couchi there is a focussing biconvex lens. All of the euphausiid photophores are well innervated. Usually, in each photophore is a nerve that branches among the photogenic cells.

An interesting characteristic of photophores was detected in Meganyctiphanes norvegica (Hardy, 1962). The luminous organs of M. norvegica, located on the optic stalks, the thorax, and the abdomen, are mobile and change their position depending on the direction of incident light. The movements of the photophores accord with those of the eyes, both taking place in the same direction. The eyes of M. norvegica rotate in the sagittal plane at an angle of 90°; the thoracic and abdomenal photophores can rotate 180° forward, downward, and backward. When Euphausiacea are illuminated by red light, the photophores direct themselves downward or backward. When the light source is shadowed, the photophores are directed forward. /24

Decapoda--Of the Decapoda, mainly the common shrimp luminesce. The luminescence of shrimp is extracellular or intracellular. In some (Systellaspis), both kinds of luminescence are combined. However, a release of slime has never been observed simultaneously with intracellular luminescence in these shrimp (Nicol, 1960a). Accordingly, the luminous organs of Decapoda are quite diverse in structure. Among them, a transition from a simple aggregate of photogenic cells (extracellular luminescence) to photophores of complex structure resembling the organs of luminescence of molluscs and Euphausiacea (intracellular luminescence) is observed.

Extracellular luminescence has been detected in some species of the genera Plesiopenaeus, Heterocarpus, Pandalus, Systellaspis (together with intracellular). The luminous organs of these shrimp are represented by many small glands scattered around the mouth and at the base of appendages. Each such gland is formed by a group of eosinophilic cells that release a secretion into a small cavity from which the luminescent slime is ejected through a narrow duct to the outside. Intracellular luminescence of specialized photophores occurs in representative of the families Penaeidae, Hoplophoridae, and Sergestidae. The number of photophores in shrimp can be very great (up to 150 in Sergestes challengeri, S. prehensilis). Usually, they are scattered all over the body, but arranged so that the light they radiate is directed downward. The structure of photophores differ. Five kinds of photophores have been found in shrimp with intracellular luminescence (Dennell, 1940).

Fish--Luminescence is widely disseminated among fish, especially among the denizens of deep waters. About 98% of all deep-water fish have photogenic organs of one kind or another (Haneda, Johnson, 1962a). Near the Bermuda Islands at depths from 700 to 5,500m, 39% of all fish families, 81% of all genera, 66% of all species, and 96.5% of all individuals luminesce (Beebe, 1937). In the opinion of N. Marshall (1954), two thirds of all deep-water pelagic fish species are luminescent.

All three kinds of luminescence occur in fish: extracellular, intracellular (fig. 12), and that due to symbiotic luminous bacteria. True extracellular luminescence characterized by the release of luminescent slime secreted by specialized cells has been detected in Searsia koefoedi and S. schnakenbecki (Nicol, 1958a). A bright blue-green cloud will appear around Searsia when the fish is taken in the hand. Skin glands produce the luminescent secretion in Searsia. The release of luminescent slime in other fish observed in some cases (for example, Malacocephalus laevis) is not a true extracellular luminescence, since this slime is an aggregate of luminous symbiotic bacteria ejected from special cavities on the fish's back. /25

A distinctive sign of fish with intracellular luminescence is bright large photophores, sometimes quite numerous. The simplest constructed photophores are found in Chondrostei. The photophores of the shark Spinax niger are cup-shaped epidermal protuberances, 100-300 microns in size. Part of the cells of the epidermis within such photophores plays the role of photogenic cells, another part--the role of lenses. The photophore has a diaphragm consisting of pigment cells.

The skin photophores of deep-water bony fishes--recesses in the epidermis--are filled with photogenic cells. The pigment layer lining the bottom of the recess acts as reflecting screen. Often, fish photophores contain additional reflectors and lenses representing modified muscle fibers. The photophores of deep-water fish are of both closed and open types. The structure of both types of photophores is similar. They differ only in that the open-type photophores have a special duct opening to the outside. Photophores of both types have been detected in some fish.

The suborbital photophores of Stomias brevibarbatus, S. richardsoni, S. ferox, Photostomias guernei, Idiacanthus fasciola, Chauliodus sloani, and Astronesthes elucens have a spherical or oblong shape and consist of a mass of photocytes--small polygonal cells, sometimes elongated, arranged radially. The photocytes contain acidophilic granules. The photophores have two membranes of which one, the inner, sometimes acts as reflector, while the second, a black pigmented one, serves as screen (Nicol, 1960b). In many deep-water fish, the photophores are located on barbels. Fish with luminescent barbels occur in the families Melanostomiatidae, Stomiatidae, and Astronesthidae. Photophores on barbels help the fish attract prey (Marshall, 1954). Fish photophores are usually well innervated.

Recently, a new kind of luminescent system in fish, located inside the body and connected with the gut, was discovered (Haneda, Johnson, 1962a,b). The new-type organ of luminescence was found in a fish inhabiting medium depths--Parapriacanthus ransonneti (family Pempheridae). In P. ransonneti, the photogenic organ, represented

by two glands, is located inside the body. The anterior or thoracic gland has a U-form and is connected to a pair of pyloric appendages. The second (anal) gland is in the posterior part of the rectum and opens to the outside through a small aperture next to the anus. Another species of the same family, Pempheris klunzingeri, has an organ of luminescence of about the same structure, but in this case the first pair of pyloric appendages is transformed into a U-shaped gland (Haneda, et al., 1966). /26

The organs of luminescence of Apogon ellioti and Siphamia majimai (family Apogonidae) belong to the new type of luminescent system described above. A. ellioti also has a luminous organ in the form of thoracic and anal glands. The thoracic gland--lemon yellow in color--opens into the second flexure of the gut. The two anal glands resemble two beans imbedded in the muscle tissue on both side of the rectum, immediately in front of the anus. The glands are not connected to the pyloric appendages. All of the described organs were composed of gland cells filled with eosinophilic secretion (Haneda, Johnson, Shimomura, 1966). The luminous organ of Siphamia majimai consists of photogenic cells representing alveolar glands with granular secretion and of a duct opening into the gut (Iwai, 1958). The luminous organs also contain transparent muscle fibers that act as lenses, and reflectors that are opaque. These new type organs are open.

Closed-type photogenic organs, not connected to the gut, but with an unusual structure, were recently discovered in several species of fish of the genus Lestidium (family Paralepididae) (Haneda, 1958b, 1964). The luminous organs of Lestidium polixum, L. elegans, and L. atlanticum are a long yellow body extending along the medial line in front of the anus. The organ is filled with a yellowish substance that is topped with transparent muscle that acts as a lens. In L. japonicum, L. pofi, and L. intermedius, the luminescent organs are paired. They lie on both sides of the medial line and join in front of the anus.

The third kind of luminescence, i.e., that due to symbiotic bacteria, has been detected in bony fish belonging to eight families: Macrouridae, Gadidae, Monocentridae, Anomalopidae, Acropomatidae, Leiognathidae, Saccopharyngidae, Trachichthyidae (Harvey, 1957a; Kuwabara, 1955). Mainly, these fish inhabit medium depths. Their luminous organs are the open type--they open to the outside through special ducts. Such organs consist of cavities of different shape filled with glandular tissue in which the bacteria live. The bacteria-containing cavities are surrounded by reflectors; in some instances, the bacterial luminescence is focussed by lenses of modified tissue, most frequently muscle. The luminescent glands in Macrouridae, Gadidae, Acropomatidae, and Leiognathidae are situated on the /27

ventral side of the body. Slime with the luminous bacteria can be ejected to the outside through a duct opening into the rectum. In Coelorhynchus japonicus, Physiculus japonicus, and Hymenocephalus striatissimus, the luminous organs are located beneath the skin; in Monocentris japonicus, the luminous bacteria live in a cavity beneath the mandible; the organs of luminescence of Gazza and Leiognathus ring the oesophagus, with which they are connected by a duct. In Acropoma japonicum, the luminous organ lies in the musculature in the ventral part of the body and has a long duct opening to the outside.

The well-known luminescent fish Photoblepharon palpebratus and Anomalops katoptron have a large, brightly luminous organ beneath each eye. The inner cavity of this organ is filled with epithelial tissue containing rod-shaped luminescent bacteria. The tissues surrounding the light organ of these fish often transform into reflectors and lenses, as in the photophores of deep water fish.

The luminescence of the Australian fish Cleidopus gloria-maris also belongs to the third type of luminescence (Haneda, 1966). C. Gloria-maris has two elliptical luminous organs located on both sides of the mandible. The surface of the orange-reddish organ is topped with a transparent colorless tissue containing many black pigmented chromatophores. The luminous organ of C. gloria-maris contains a self-luminescent body, has a duct, reflector, lens, and orange-reddish filter. The luminescent body is an aggregate of gland cells. The luminescence of this fish is a continuous bluish-green color. It is interesting that the organ itself gives off a bluish color. The greenish luminescence occurs after passage through the orange-reddish filter. When the fish closes its mouth, the maxilla covers the luminous organ and the luminescence disappears. In the gland cells of the light organ live symbiotic luminous bacteria that can be isolated in a pure culture.

Photobacteria have been isolated from luminous organs of almost all fish of the above mentioned families. In the appropriate nutrient medium, pure cultures of these bacteria display luminescence of different degrees of brightness. It was impossible to obtain luminescence only in bacteria isolated from the luminous organs of Anomalopidae. The luminous bacteria symbionts will be examined in more detail in Chapter III.

It is interesting to note that luminescence due to symbiotic bacteria can suddenly be discovered in families in which all representatives have intracellular luminescence. Thus, Y. Haneda in 1965 reported symbiotic luminescent bacteria found in the inner part of the luminous organ of Siphamia versicolor (family Apogonidae) that opens into the gut. Another close species, S. majimai, has intracellular luminescence. The eggs and larvae in the early stages of

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development of S. versicolor do not luminesce. It may therefore be supposed that the luminous bacteria get into the luminous organ later.

BIOLUMINESCENCE AS A CHEMILUMINESCENT REACTION CATALYZED BY AN ENZYME. BIOCHEMISTRY OF KNOWN LUMINESCENT SYSTEMS OF MARINE ORGANISMS.

Bioluminescence is a chemiluminescent reaction catalyzed by an enzyme. It is known that many chemical processes have exothermal stages. In such reactions, molecules with excess energy, i.e., found in disequilibrium excited states, are usually formed or are present. Deexcitation is achieved in different ways, including by light radiation--chemiluminescence. An example of chemiluminescent reaction is the oxidation reaction of organic substances, for example the oxidation of hydrocarbons, alcohols, aldehydes, amines.

The entire process of chemiluminescence can be divided into two stages: (1) formation of the excited product P* from initial reagents K and M: $K + M \rightarrow P^*$; (2) transition of the excited molecule P* to the ground state with the emission of a light quantum: $P^* \rightarrow P + h\nu$ (Reed, 1960). From this, two basic questions arise in the study of chemiluminescent reactions: (1) in what stages of the reaction are the excited molecules formed; what factors determine the effectiveness of excitation, (2) how and how effectively is the excitation energy converted into radiation.

Bioluminescence is enzymatic chemiluminescence, since the excited states of luminescing molecules in the luminescence of living organisms are produced by the oxidation of the luciferin substrate in the presence of the specific enzyme, luciferase.

In the case of bioluminescence, the first question arising in the study of chemiluminescent reactions (see above) in essence boils down to two problems: (1) the need to identify the excited and the emitting molecule, which is very hard to do even in the simplest luminescent systems and which is also complicated by the possible migration of energy from excited molecule to an emitting one; (2) explanation of those stages of a chemiluminescent reactions that yield energy for light emission. Very little has been done in the last direction either. Spectroscopic investigations of the bioluminescence of different organisms have shown that quite high energies are required for light emission--from 41 kcal (in the red region) to 62 kcal (in the blue region) per mole of substance that undergoes chemical changes with light emission. These values considerably exceed the energy capacity in 10-20 kcal/mole which an organism usually has available (for example, the energy supply of high-energy phosphate bonds). However, such amounts of energy, according to S. Reed (1960), can be obtained by the accomplishment of successive reaction stages, each of which requires a small amount

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of energy. The need for large energies for luminescence processes also leads to the idea that luminescent reactions in living organisms must be connected with the main channel of energy liberation in a cell--the respiratory chain. Such a connection has actually been established in luminescent bacteria.

To date, the question of the efficiency of the transition of energy from the excitation of the molecule to radiation in a bioluminescent reaction has been little studied, although quantum efficiency is the main indicator of all chemical reactions, including light absorption or emission. In the case of bioluminescence, the major difficulty in determining quantum efficiency is in obtaining highly purified components of the luminescent reaction and in reproducing this reaction in vitro. Quantum efficiency has only been determined in three bioluminescent reactions--the firefly Photinus pyralis, the crustacean Cypridina, and luminous bacteria. The highest quantum efficiency, equal to unity, is found in the bioluminescent reaction of the firefly. For each oxidized molecule of firefly luciferin, a light quantum (more precisely, 0.88 ± 0.12) is emitted (Seliger, McElroy, 1960). This high value of quantum efficiency is unique and substantially exceeds the quantum efficiency of nonbiological chemiluminescent reactions in water solutions. For example, the highest quantum efficiency of chemiluminescent reaction detected in luminol reacting in water is one light quantum emitted for about 50 molecules of luminol oxidized (Seliger, 1961).

For one molecule of Cypridina luciferin oxidized in the reaction process, 0.29 ± 0.15 light quantum is emitted, i.e., one light quantum is generated in the oxidation of 3.4 molecules of luciferin (Johnson, et al., 1962). The quantum efficiency of the luminescence of Cypridina is the same with respect to oxygen as it is to luciferin.

In luminous bacteria, quantum efficiency is determined with respect to aldehyde--the additional component necessary for this luminescent reaction. In extracts of luminous bacteria, one light quantum is emitted per 20 molecules of aliphatic aldehyde (Cormier, Totter, 1957).

The explanation of bioluminescence as a chemiluminescent reaction catalyzed by an enzyme appeared at the end of the 19th century, thanks to the studies of the French scientist R. Dubois. Studying the luminescence of the beetle Pyrophorus, Dubois attempted to isolate the component responsible for the luminescence. To this end, he obtained extracts from the luminous organs of Pyrophorus with cold and hot water. The extract obtained with cold water, unlike that obtained with hot water, luminesced, if even for a very short time. When a cooled hot-water extract was added to the fading extract, the luminescence was renewed. On the basis of this, Dubois suggested that the luminescence of the beetle Pyrophorus is the result of the

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interaction of two substances, one of which is inactivated at high temperature, while the second is thermally stable. Later, after analogous experiments with the mollusc Pholas dactylus, Dubois called the thermally stable component of the luminescent reaction--luciferin, and the thermally labile component--luciferase. He assumed that luciferin was the substrate, while luciferase was the catalyzer of the luminescent reaction. Since luminescence was only observed in the presence of oxygen, Dubois considered this process oxidation. Consequently, the oxidation nature of bioluminescence was confirmed, even though oxygen is not always a necessary component of a luminescent reaction. Thus, among inhabitants of the sea, organisms are known which do not require oxygen for luminescence. These are the jellyfish Pelagia, Aequorea, and Halistaura, the Ctenophora Mnemiopsis, Beroë, and Eucharis, and the Radiolaria Thalassicola and Collozoum (Harvey. 1926; Harvey, Korr, 1938).

After Dubois's studies, the usual test for the presence of the substrate luciferin and the enzyme luciferase in any luminescent organism consists in obtaining cold- and hot-water extracts from the organism itself or from its luminous organ. The appearance of luminescence when these extracts are mixed attests the presence of the "luciferin-luciferase" system. In the beginning of the 20th century, Harvey worked out a method of obtaining extracts from dried luminescent organisms and detected the luciferin-luciferase reaction in the ostracod crustaceans Cypridina (Harvey, 1917b) and Pyrocypris, in the polychaetes Odontosyllis phosphorea and O. enopla (Harvey, 1921-22), in the shrimp Systellaspis (Harvey, 1921), the fireflies Photinus, Photurus, and Luciola (Harvey, 1916, 1927). To this list, several other luminescent organisms can be added in which students and followers of Harvey have detected the luciferin-luciferase reaction. These are the dinoflagellate Gonyaulax polyedra (Hastings, Sweeney, 1957a), two fish species, Parapriacanthus ransonneti (Haneda, Johnson, 1958) and Apogon ellioti (Haneda, Johnson, Sie, 1958), the lamelbbranch Rocellaria grandis (Haneda, 1939), the polyp Renilla (Cormier, 1960), the shrimp Heterocarpus sibogae (Haneda, 1955), the luminous fungi Collybia and Armillaria (Airth, Foerster, 1960). Luciferin and luciferase were not detected in jellyfish, comb jellies, nemertines, Cephalopods, Copepods, Myriapods, Ophiuroidea, and Tunicata. /31

In pure form, luciferin and luciferase have been isolated from a still smaller number of organisms. The substrate and enzyme of the luminescent reaction have been isolated from the ostracod crustacean Cypridina hilgendorfii, the firefly Photinus, the fish Apogon ellioti and Parapriacanthus ransonneti; only luciferase has been isolated from luminous bacteria. Recently, luciferin and luciferase were obtained from the luminous organs of the deep-sea shrimp Hoplophorus gracilorostris (Johnson, et al., 1966).

Notwithstanding the limited number of organisms from which the substrate and enzyme of luminescent reaction have been isolated, it is already possible to state that the luciferins and luciferases in the various species have different structure and a high specificity (with a few exceptions). Moreover, the luminescent reactions of marine organisms also differ in the degree of complexity. Complex reactions occur as the result of the inclusion of supplementary components (besides luciferin, luciferase, and oxygen), such as ATP; pyridine, flavin, and adenine nucleotides; peroxides; and aldehydes; and the related occurrence of intermediary stages preceding the actual reactions of light formation.

The simplest luminescent system was recently discovered in the Hydromedusae Aequorea aequorea and Halistaura. The luminescence of these organisms in vitro is observed when calcium or strontium ions are added to the proteins isolated from jellyfish extracts (aequorin in the case of Aequorea, and halistaurin in the case of Halistaura). The reaction proceeds in the absence of oxygen (Shimomura, Johnson, Asiga, 1962, 1963a,b).

The light-emitting reaction in crustaceans (Cypridina), flagellates (Convaulax), helminths (Odontosyllis), and fish (Parapriacanthus) includes a substrate (reduced luciferin), an enzyme (luciferase), and oxygen, and follows the general scheme $LH_2 + O_2 \rightarrow Ehv$, where LH_2 is the reduced luciferin, E is the luciferase. More complex, multicomponent, luminescent reactions that proceed in several stages have been detected in luminous bacteria, sea pens, Enteropneusta, and insects (Lampyridae). The efforts of biochemists studying luminescence are, at present, chiefly directed to the identification of the reaction components, the determination of the stages immediately preceding light emission, and to the delineation of the intermediate or end reaction products responsible for the luminescence.

Only a small number of luminous species from the total number of bioluminescents in the sea have become the object of such biochemical studies. Nonetheless, it is now already possible to assume that the total number of types of luminescent reactions (characterized by the participation of similar components and inclusion of like intermediate stages) is not great in marine organisms.

Types of Luminescent Reactions of Marine Organisms: /32

I. Aequorea, Halistaura } Protein $\xrightarrow{Ca^{++}}$ hv (oxygen not required)

II. Gonyaulax $LH_2 + \frac{1}{2} O_2 \xrightarrow[\text{inorganic salt}]{\text{luciferase}} L + H_2O + hv$

Odontosyllis, Pholas, Cypridina, Parapriacanthus, Apogon } $LH_2 + \frac{1}{2} O_2 \xrightarrow{\text{luciferase}} L + H_2O + hv$

III. Renilla $LH_2 + \frac{1}{2} O_2$ + 3'5'-diphosphoadenosine $\xrightarrow{\text{luciferase}}$
$\rightarrow L + H_2O$ + products? + hv

Bacteria $FMN\text{-}H_2 + \frac{1}{2} O_2$ + aldehyde $\xrightarrow{\text{luciferase}}$
$\rightarrow FMN + H_2O$ + products? + hv

IV. Balanoglossus $LH_2 + H_2 O_2 \xrightarrow{\text{luciferase}} L + 2H_2O + hv$

Four types of luminescent reactions found in marine organisms are presented above. It appears that the type of luminescent reaction is not connected with the level of organization of the bioluminescent. Thus, type II luminescent reaction, including only luciferin, luciferase, and oxygen, was found in organisms on different steps of the evolutionary ladder--in the flagellate Gonyaulax and crustacean Cypridina, the mollusc Pholas, and fish Apogon. At the same time, the luminescent system of photobacteria differs considerably by virtue of great complexity.

Let us examine in more detail the best studied luminescent systems of marine organisms (see Chapter III on luminous bacteria).

Cypridina hilgendorfii. The luminescent system of ostracod crustacean Cypridina hilgendorfii belongs to type II. To luminesce, only the substrate luciferin, the enzyme luciferase, a solvent (water), and oxygen are required (Harvey, 1952, 1953). The luciferin and luciferase are formed as granules in the luminescent glands. The large granules (to 10 microns) contain the luciferin, the small granules (to 2 microns) the luciferase. The luminescent reaction occurs in seawater into which both kinds of granules are ejected.

The luciferin-luciferase reaction in Cypridina hilgendorfii was first demonstrated by Harvey (1916, 1917b) working with unpurified water extracts from dried crustaceans. Unpurified luciferin from such extracts was first obtained by R. Anderson (1936). Later, important investigations were conducted of the luminescent system

of this crustacean (Harvey, 1952, 1953; Harvey, Tsuji, 1954; Chase, 1949a, b, 1960; Mason, 1952; Tsuji, 1955, and others). Shimomura and colleagues, using a method of purifying luciferin proposed by Anderson, obtained crystalline luciferin (Shimomura, Goto, Hirata, 1957). The crystallization method was later improved and simplified (Haneda, et al., 1961).

The luciferin of C. hilgendorfii is a low-molecular compound of yellow color with acid-base (amphoteric) properties. It dissolves in the lower aliphatic monohydric alcohols and has a redox potential slightly less than that of the hydroquinone-quinone system.

Crustacean luciferin is a derivative of indole (Shimomura, Goto, Hirata, 1957). It was recently shown (Kishi, Goto, Hirata, 1966) that the luciferin of C. hilgendorfii, depending on the pH of the medium, can either be in the form of the hydrochloride (weak alkaline medium) or in the form of the hydrobromide (strongly acidic medium). The crystalline luciferin, obtained by O. Shimomura (Shimomura, Goto, Hirata, 1957), was the hydrochloride. Kishi (Kishi, Goto, Hirata, 1966) obtained crystalline luciferin in the form of the hydrobromide. Results of an elementary analysis of luciferin hydrobromide agrees well with the formula $C_{22}H_{27}ON_7 \times 2HBr$, while the formula $C_{22}H_{29}O_2N_7 \cdot 2HCl$ is more acceptable for luciferin hydrochloride.

The oxidation of luciferin and the formation of oxyluciferin (oxidized form of luciferin) can be represented by the following structural formulas (Kishi, Goto, Hirata, 1966):

luceferin hydrobromide (colorless)

luciferin hydrochloride (reddish color)

oxyluciferin

The luciferase of C. hilgendorfii was first isolated in pure form in 1951 (McElroy, Chase, 1951). By means of column chromatography a highly purified luciferase was obtained (Shimomura, Johnson, Saiga, 1961). This luciferase is pale pink; its absorption maximum is typical of proteins, about 277 millimicrons. Possibly, the luciferase of C. hilgendorfii is a metalloprotein. The molecular weight of luciferase is between 48,500 and 53,000; the isoelectric point was found to be at pH 4.34. The greatest enzyme activity is observed at pH 7.3 (Shimomura, Johnson, Saiga, 1961). It had been shown earlier that the maximum luciferase activity is at 24°; the enzyme activity falls off rapidly in both sides of the maximum (Chase, Lorenz, 1945). [24]

The luminescent reaction of Cypridina is easily obtained in vitro by mixing luciferin and luciferase solutions at appropriate temperature and pH values (Johnson, Shimomura, Saiga, 1961). The minimum amounts of luciferin and luciferase that give luminescence discernible to the dark-adapted eye when mixed, are 10^{-5} µg/ml of luciferin and 0.01 µg/ml of luciferase (or 1 µg/ml of luciferin and 10^{-7} µg/ml ($2 \cdot 10^{-15}$M) of luciferase). Crustaceans weighing about 4mg (dry weight) contain 1 µg each of luciferin and luciferase (Johnson, Shimomura, Saiga, 1961).

When luciferin and luciferase are mixed (fig. 13) in the presence of oxygen, the luminescence intensity increases to maximum considerably slower than when degasified luciferin and luciferase solutions are mixed with water containing dissolved oxygen (Chance, Harvey, Johnson, Millikan, 1940).

The luminescent reaction of Cypridina in vitro proceeds like a first order reaction; its kinetics can be described by the Michaelis-Menten equation for a reaction with the formation of an intermediate enzyme-substrate complex (Chase, Harvey, 1942; Chase, 1960). The Michaelis constant is $5.95 \cdot 10^{-7}$M (Chase, 1949b). In experiments with well refined luciferin and luciferase, $K_m = 5.2 \cdot 10^{-7}$M (Shimomura, Johnson, Saiga, 1961). [25]

It is still not clear what molecule emits light in the luminescent reaction of Cypridina. Experiments (Harvey, 1924) in mixing the luciferin and luciferase of crustaceans luminescing blue and yellow light attest that the emitting molecule was the luciferase (the radiation color was determined by the luciferase). However, later, when crystalline luciferin had been obtained, its absorption spectrum in the visible region was found to be identical to the emission spectrum of the luminescent reaction (Shimomura, Goto, Hirata, 1957; Harvey, Chase, McElroy, 1957). On this basis it was assumed that in the luminescent reaction of Cypridina, which is catalyzed by luciferase, the luciferin molecule is excited and emits a light quantum (Johnson, Eyring, Chang, 1959).

In the absence of luciferase, luciferin undergoes nonluminescent oxidation, which may be accelerated under the action of ferricyanides and some other inorganic agents. According to spectroscopic data, the end products in the nonluminescent oxidation of luciferin do not differ from the products of enzymatic oxidation (Chase, 1940). However, the difference consists in that the products of nonenzymatic oxidation can be converted into active luciferin by such reducing agents as $Na_2S_2O_4$, while the luminescent oxidation process is irreversible (Tsuji, 1955).

Luminescent and nonluminescent oxidation of luciferin can be presented in the following form:

$$LH_2 \begin{cases} + \frac{1}{2} O_2 \rightarrow L + H_2O \\ + A + \frac{1}{2} O_2 \rightarrow L + H_2O + h\nu \end{cases}$$

where LH_2 is luciferin, A is luciferase, L is oxyluciferin. We spoke about the quantum efficiency of luminescent reaction in vitro above. 36

Fish--To date, the luminescent systems of only two fish species, Parapriacanthus ransonneti and Apogon ellioti have been investigated. Extracts from the luminous organs of these species display a typical luciferin-luciferase reaction. Luciferin and luciferase were first isolated from the luminous organs of these fish in 1958 (Haneda, Johnson, 1958; Haneda, Johnson, Sie, 1958). The luciferin of P. ransonneti was obtained in crystalline form in 1961 (Johnson, Sugiyama, 1961). The luminescent reaction of these fish is simple (type II) and analogous to that of Cypridina hilgendorfii. It only involves luciferin, luciferase, and oxygen. In a chemical investigation of the reaction components, the similarity in the luminescent systems of the fish and the ostracod crustacean was found to be even deeper. Thus, crystalline luciferin of P. ransonneti does not differ from the luciferin of C. hilgendorfii with respect to chemical properties. The total identity of fish and crustacean luciferins was also revealed in comparing their absorption spectra. It is not surprising, therefore, that the luciferin of P. ransonneti produced luminescence with the luciferase of C. hilgendorfii, while the luciferin of the crustacean luminesced with the fish luciferace. Reciprocal cross luminescent reactions are also observed between P. ransonneti and A. ellioti as well as between A. ellioti and C. hilgendorfii. The emission spectra of all these luminescent reactions are also identical. However, fish and crustacean luciferases differ with respect to several properties. Thus, recent investigations (Tsuji, Haneda, 1966) have shown that the A. ellioti luciferase increases activity when diluted or kept in a phosphate buffer, while the activity of C. hilgendorfii luciferase falls off under these conditions. These enzymes also differ with respect to immunological properties.

The identity of fish and crustacean luciferins gave reason to assume that fish luciferin owes its origin to crustacean luciferin. The following facts support this hypothesis (Haneda, Johnson, Shimomura, 1966):

In the ventricles of many P. ransonneti (of 2,300 samples studied) dead crustaceans were found continuing to luminesce. When illuminated by ultraviolet light, the pyloric appendages and the stomach of R. ransonneti produce a bright yellow-green fluorescence (fig. 14), indicating the presence of luciferin. Pure luciferin, isolated from the pyloric appendages of this fish, was identical to that of C. hilgendorfii.

The geographic distribution of C. hilgendorfii does not coincide with that of P. ransonneti and A. ellioti. However, in the regions these fish inhabit, another luminescent ostracod crustacean, C. noctiluca, is often found. It is quite apparent that it is still impossible to resolve the question of the origin of fish luciferin on the basis of available facts. Y. Haneda believes that only when fish are cultivated under conditions that exclude C. hilgendorfii or C. noctiluca as a food source will it be possible to determine whether these fish synthesize luciferin themselves or obtain it ready-made from the crustaceans.

Gonyaulax polyedra--The luminescent system of this photosynthesizing flagellate also belongs to type II.

The biochemical bases of G. polyedra light emission are somewhat unusual. Side by side with the classical luciferin-luciferase reaction displayed when noncellular extracts are mixed, the luminescence of scintillons (crystalline granules), which had precipitated out of the decomposed cells by centrifugation, could be observed in the flagellate. The scintillons do not produce a luciferin-luciferase reaction.

The luciferin-luciferase reaction of G. polyedra was first demonstrated in noncellular extracts (Hastings, Sweeney, 1957a). For this luminescent reaction to occur, inorganic salts (for example, NaCl) in quite high concentrations are necessary in addition to the substrate, enzyme, and oxygen. When sodium chloride is added to a luciferin-luciferase mixture, notable changes are observed in the configuration of the enzyme molecule. It is assumed that these changes are caused by increased luciferase activity. G. polyedra luciferin and luciferase has recently been obtained in a partially purified form (Hastings, Bode, 1961).

As far as the scintillons are concerned, they are now being studied sufficiently well. Electron-microscopic investigations have shown that they are typical rhombohedrons, single or paired, possessing strong birefringence. Their size ranges from 0.1 to 0.6 micron (see fig. 2). In the presence of oxygen and when the pH factor of the medium lowers, scintillons produce flashes similar to those of the living organisms. The optimal luminescence of these particles is observed at pH 5.7, while for a G. polyedra luciferin-luciferase reaction, the optimum is at pH 6.8. It had originally been assumed that scintillons were an independent bioluminescent system, distinct from the luciferin-luciferase system (De Sa, Hastings, Vatter, 1963). However, investigations of recent years have shown that this is not the case. Direct proof has been obtained that when scintillons are decomposed by acid, luciferase is liberated. The oxygen requirement for both scintillon luminescence and the luciferin-luciferase reaction, as well as a certain similarity in the spontaneous inactivation of scintillons and luciferin, give reason to believe that luciferin enters into the composition of scintillons (Hastings, et al., 1966). J. Hastings suggests that in vivo, G. polyedra flashes are caused by hydrogen-ion transport from the cytoplasm to the scintillons. In vitro, this is achieved by lowering the pH and raising the hydrogen-ion concentration. For the G. polyedra luminescent reaction, Hastings proposes the following model: the luciferase and the luciferin anion form a stable configuration in the scintillon structure at definite pH values (7.5-8.5) within the scintillon membrane. When there is excitation, the permeability of the membrane for protons increases. Protons passing through the membrane react with the luciferin anion and form an active luciferin which oxidizes rapidly, producing a flash of light. The flash intensity of one scintillon is proportional to the number of protons transported through the membrane. Apparently, luciferin can also be found directly in the cytoplasm. It does not react with the luciferase here, probably because of an inadequate concentration of hydrogen ions or salts.

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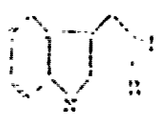
Odontosyllis enopla--The fact that the luciferin-luciferase reaction in this polychaete worm also belongs to the type II luminescent systems has been known since 1921 (Harvey, 1921-1922). However, for a long time there was no new information on the luminescence of O. enopla. Only in 1963 were partially purified luciferin and luciferase obtained and some properties of the bioluminescent system determined (Shimomura, Beers, Johnson, 1964). It was found that the optimum pH for a luciferin-luciferase reaction was at 6.8-7.2. Within these pH limits, the initial reaction rate can be increased by adding small amounts of cyanide, the total amount of emitted energy remaining unchanged. Recently, incompletely purified preparations of luciferin and luciferase were isolated from frozen and dried worms (Seliger, McElroy, 1965).

The luminescent system of this worm has an interesting peculiarity: After luciferase has been added to the luciferin solution, a definite lag phase (or latent period) is observed: the luminescence does not reach maximum intensity immediately. Unfortunately, nothing is known of the time characteristics of these reactions. It has also been established that the maximum of luciferin fluorescence coincides with the bioluminescent emission peak (540nm). Since the luciferin fluorescence decreases during the luminescent reaction, Seliger and McElroy postulate that O. enopla luminescence is a sensitized chemiluminescence (Seliger, McElroy, 1965).

Renilla reniformis--The luminescent system of these colonial Coelenterata belongs to type III. Active extracts of R. reniformis luciferin and luciferase were first obtained by M. Cormier (Cormier, 1960, 1961), who also evaluated some characteristics of this bioluminescent system. It was found that, calcium ions and adenine nucleotide also are involved in addition to the substrate, enzyme, and oxygen. This nucleotide is 3.5-diphosphoadenosine (DPA) (Cormier, 1962). Recently, purified luciferin was obtained and its structure determined (Cormier, Hori, Kreiss, 1966). The substrate of the luminescent reaction of this coelenterate is a derivative of triptamine and has the following structure:  [39]

The luminescent reaction in the presence of DPA proceeds in two stages. The first stage includes the activation of luciferin by 3.5-diphosphoadenosine and the formation of an intermediate complex in the presence of calcium ions. In the second stage, the activated luciferin begins emitting in the presence of luciferase. A luminescent reaction can also proceed in vitro without DPA. Simple heating of the luciferin in an acidic medium leads to the formation of the active product, which when mixed with luciferase under aerobic conditions produces luminescence (Hori, Yamadi, Cormier, 1967). A similar phenomenon is also observed in the indican-peroxidase chemiluminescent system of horseradish. By heating at low pH values, indican can be transformed into the more active indoxyl which reacts with peroxidase in the presence of H_2O_2 with light emission.

The luciferin and luciferase of Renilla reniformis, R. mülleri, and R. köllikeri produce luminescence in cross reactions, attesting to a certain similarity in the luminescent systems (Cormier, Eckroade, 1962).

Balanoglossus biminensis--A unique kind of luminescent system (type IV) has recently been discovered in a representative of the Enteropneusta, Balanglossus biminensis (Dure, Cormier, 1961). For this organism to luminesce, peroxide is required in addition to luciferin, which is of a protein character, and luciferase.

Hydrogen peroxide, benzene peroxide, as well as such peroxide-forming systems as glucose oxidase and L-aminooxidase can serve as the peroxide. It is possible that the luciferase of the balonglossid itself has the properties of a peroxidase.

II. BIOPHYSICAL CHARACTERISTICS OF THE LUMINESCENCE OF MARINE BIOLUMINESCENTS

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STIMULATION OF LUMINESCENCE

Continuous uninterrupted luminescence is only known for bacteria, fungi, Myriapoda, and certain insects. The luminescence of other organisms occurs in separate flashes that arise spontaneously or through stimulation. Spontaneous flashes are sometimes noted in Ctenophora (Bolinopsis and Pleurobrachia), in the euphausiid Meganyctiphanes norvegica, and in some fish. The luminescence of Protozoa, most Coelenterata, helminths, Copepoda, molluscs, and fish having independent luminescence only occurs under stimulation. Under natural conditions, the luminescence of these organisms is stimulated by mechanical vibrations (sea turbulence, ship movement, or movement of large animals). It is possible that the stimuli for luminescence in nature may also be other factors--electrical and mechanical fields, thermal gradient, light (including that of biological origin), or chemical compounds. The investigation of natural stimuli is one of the main ways of understanding the biological role of bioluminescence in the sea. Unfortunately, very little has been done along these lines.

In the case of extracellular luminescence, stimulation leads to a release of a luminous secretion either via a neuromuscular mechanism or directly via the nervous system. In the former case, luminescent glands or special secretion-containing cavities are compressed when excited by external stimulation by means of muscle fibers, as a result of which the secretion is ejected outside (Cypridina, Copepoda, Systellaspis). This is an indirect type of luminescence regulation or control. In the latter case, the luminescent glands are well innervated and are excited themselves under stimulation. Here, external stimulation causes luminescence through nerve regulation of the secretion of luminous material (direct type of control). The direct type of control of extracellular luminescence has been detected in some Coelenterata, helminths, and molluscs. In the case of intracellular luminescence, the stimulation causes physicochemical changes in the excited photogenic cells, as a result of which a luminescent reaction occurs. Finally, the stimulation can regulate luminescence through the inclusion of mechanisms that bring into operation the screens and diaphragms of the light organs. This kind of luminescence regulation is accomplished directly through the nervous system. It is found in molluscs and fish having complex photophores or light organs with symbiotic luminous bacteria.

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It must be said that much remains unclear about how external stimulation results in a flash of light. In multicellular organisms, luminescence usually occurs after tactile stimulation or stimulation of the photoreceptors. As a rule, this is a reflex process. In the sea pens, Pennatula, propagation of the luminescence wave over the entire colony is observed when mechanical stimulation is applied at any point. Tactile stimulation of Renilla also results in luminescence of the entire colony (Nicol, 1960a). In the polychaete Chaetopterus, the luminescent response to tactile stimulation is very localized and when an individual segment is stimulated, luminescence is only observed in that part of the body. In Pyrosoma, luminescence will at first appear only where the mechanical stimulation has been applied, then it will spread throughout the entire colony. It is interesting that flashes in one colony can cause luminescence in other colonies. Thus, light as well as mechanical stimulation can be a natural stimulus luminescence in pyrosomids. Luminescence resulting from tactile stimulation has been observed in many fish with well developed photophores (Myctophum, Maurolicus, Cryptopsarus).

The ability of many marine bioluminescents to respond to stimulation by flashing makes it possible to study their light signals under laboratory conditions and to determine the characteristics of bioluminescent pulses: amplitude (intensity), latent period, and duration, as well as the spectral distribution of the radiation. Such investigations facilitate an understanding of conditions of generation of light impulses when stimulation is applied and to approach the solution of the question of luminescent reaction initiation and the mechanism of light pulse formation. To investigate marine organism luminescence under laboratory conditions, usually chemical, mechanical, or electrical stimulation is used. By its nature mechanical stimulation is closest to natural stimuli.

In chemical stimulation, the conditions of luminescence emission are, of course, remote from natural conditions. However, to obtain information on the radiating capacity of various organisms and to study the spectral characteristics of luminescence, this kind of stimulation is usually applied. Pulse luminescence of Protozoa, Coelenterata, and Crustacea can be elicited by the addition of formalin, ammonia solution, ethyl alcohol, iodine in low concentrations (dilutions 1:100, 1:50, 1:20, 1:10), as well as by fresh water. As the concentration of the chemical stimulus is increased, the pulse luminescence is replaced by unbroken, continuous luminescence that lasts for a period of time and then is extinguished at the death of the animal. [42]

The most complete information on the ligh flashes of various organisms under laboratory conditions can be obtained through electrical stimulation. The possibility of graduating electrical pulses precisely by parameters and of fixing the time the stimulus

has been applied makes it possible to study the conditions of light flash origin and flash characteristics. Visible light, which usually suppresses the luminescence of Protozoa and many Coelenterata, can also stimulate luminescence. Thus, flashes are observed in the euphausiid Meganyctiphanes norvegica when a tray of specimens is suddenly illuminated (Hardy, Kay, 1964). As noted earlier, light of biological origin can also stimulate the luminescence of Pyrosoma.

It is sometimes very difficult to bring about luminescence in certain bioluminescents with conventional means of stimulation. The marine bristleworm Heterocirrus binoculatus does not luminesce under chemical, electrical, or mechanical stimulation, but it does produce a flash when immersed in fresh water (Nicol, 1960c).

LABORATORY INSTRUMENTS TO MEASURE THE CHARACTERISTICS OF BIOLUMINESCENCE OF MARINE ORGANISMS

To conduct laboratory experiments, it is first of all necessary to have an adequate amount of working material--the bioluminescents under study. During expeditions, specimens are taken with plankton nets, using the standard biological collection methods. Plankton collecting is unavoidably accompanied by the mechanical destruction of a considerable part of the bioluminescents, from the effects of temperature gradients, pressure gradients, salinity, illumination, etc. On shipboard, right up until the experiment is conducted, the plankton is kept in concentrated samples. All of these factors have an unfavorable effect on the experimental specimens, and negative results of laboratory investigations require repeated close checks.

The history of laboratory investigations of marine organism luminescence covers many decades. However, until the mid-1950's, the imperfect techniques used to study bioluminescence made possible only a qualitative evaluation of light effects (Harvey, 1952, 1957). 43
With the development of instrumental investigations of bioluminescence directly in the depths of the sea, the need arose to obtain precise information on the luminescence characteristics of organisms creating a luminescent field at one or another depth. In this connection, during the last decade several models of laboratory instruments have been developed to measure the luminescent signals of individual organisms and new methods of investigation attuned to modern technological capabilities have been proposed. In laboratory measurements, many investigators have used the photometric apparatus intended for underwater operations (Clarke, Hubbard, 1959; Hardy, Kay, 1964). The experimental organisms are put in a dark chamber that protects the instrument's photomultiplier from external illumination. Since very few bioluminescents luminesce of their own accord, the dark chambers are equipped with stimulation devices.

The installation used by David and Conover in 1961 to record crustacean luminescence used a portable bathyphotometer designed by L. Breslau. To increase instrument sensitivity, the photomultiplier signal passed through a transistor amplified. Signals were recorded at a rate ranging from 15 cm/hr (when recording flash frequency) to 15-30 cm/min (when measuring duration and energy). The luminescence was stimulated by electrical pulses. This kind of stimulation has several advantages compared with mechanical action; the intensity and duty factor of the activating pulses change within broad limits. Pulses can be fixed using any recording method with the required accuracy simultaneously with the light flash, thereby permitting estimation of the threshold excitation value, the latent period, and determination of change in the parameters of light signals in the case of successive stimulations. In order to obtain a constant value of the stimulating action from experiment to experiment, David and Conover designed a special wax cuvette (fig. 15,a). The central operating chamber was filled with cooled sea water. One or several experimental organisms were placed in the chamber. Electrical pulses from a generator were sent through carbon electrodes connected by an agar[1]

(1) The agar was made with sea water.

microfilter to the chamber. Pulse duration could be changed within considerable limits. The interval between pulses was 0.2 sec. The current through the vessel was measured with an accuracy of 0.1A. This design permitted precise determination of the duration and stimulating pulse, but the form of the electrodes creates in the cuvette an electrical field of complex configuration with unequal voltage at different points as a result of which the stimulating action (which in our opinion is proportional to current density) will differ according to the position of the bioluminescent in the cuvette. This nullifies the attempt of the experimenter to obtain a selected constant excitation value. 44

A whole series of studies on the measurement of bioluminescence under laboratory conditions were performed by Nicol (1957a, 1958a, b, c, d, e), who developed an apparatus and methods of studying the intensity and form of luminescent flashes of different organisms, the kinetics of luminescent reaction, and the spectral distribution of the emitted light. In one of the installations to record luminescent flashes, a photomultiplier with an antimony-cesium cathode with maximum spectral sensitivity in the blue-violet region was used (Nicol, 1958b). The calibration of the system to determine luminescence intensity was made with a photometric standard (Co^{60}-activated phosphor). A cathode oscillograph with photorecorder served as recorder.

The experimental animal is placed directly in front of the photomultiplier cathode in the special cuvette (fig. 15,b). Stimulation is by rectangular electrical pulses from an electronic apparatus or by discharge of a capacitor. The stimulating pulses are fixed by a second oscillograph beam. The cuvette is made of plexiglass. The bioluminescents are placed in the central chamber (1). Pulse voltage for stimulation is delivered to silver electrodes which are connected to the central chamber by agar transitions. The entire cuvette is filled with sea water and covered with a glass to prevent evaporation. Several substantial shortcomings in this design should be noted. As in the case of the design described earlier (David, Conover, 1961), a uniform field cannot be created in the working space of the chamber. As current density increases, electrolysis is noted in the cuvette, especially where the silver electrodes contact the agar transition bridges. This leads to a significant change in the total resistance of the cuvette, and, consequently, to uncontrolled change of current density in the working chamber. Nicol also used a system of mechanical stimulation that permitted the initial moment of its action to be fixed. Stimulation is accomplished by vibrating the working cuvette by the electromagnetic system of a dynamic loudspeaker. The electrical pulse to the winding of the moving coil was supplied by the charged capacitor. However, this method proved too sluggish to determine the latent period of the bioluminescent reaction of Noctiluca. Maximum speed was developed by the system only after 23ms from the beginning of the discharge, while the latent period, determined from the moment the capacitor charge ceased, was 10-30ms. [45]

Laboratory investigations of the bioluminescence of several mass plankton species have been conducted on several marine expeditions by staff members of the Laboratory of Photobiology of the Institute of Physics of the Siberian Division of the USSR Academy of Sciences. Plankton is collected using standard biological collecting methods and quantitative closing nets. Shipboard processing of the plankton samples consisted in thinning them down to a constant volume. In most cases, samples were kept in cool darkened vessels containing sea water. To stimulate light pulses, electrical, mechanical, and chemical stimuli were applied. [46]

The block diagram of the equipment for laboratory measurements of the light pulse characteristics of individual organisms is given in Figure 16. The broken line delineates the part of the equipment contained in a hermetic lightproof housing. The light signals from the bioluminescents in the cuvette are picked up by two FEU-29 photomultipliers. In front of one is a disc with eight apertures uniformly arranged around the diameter. In seven of the eight apertures are six narrow-band interference light filters and a standard photometric source consisting of a C^{14}-activated phosphor. The diameter of the light filters exceeds somewhat that of the photomultiplier cathode. The pass band of each light filter is about 10-12nm. A photometric reference standard serves to calibrate the equipment sensitivity.

Light filters are changed by manually rotating the disc. The disc aperture is precisely fixed opposite the photomultipler cathodes after each change. The photomultipliers are powered by a VS-22 stabilized high-voltage source, making it possible to regulate the supply voltage within broad limits. Recordings are made on the N-700 oscillograph. For satisfactory record quality, the pulling speed of the light-sensitive paper is 40mm/sec. Blinds, shown in the block diagram with solid lines, make it possible to change the cuvettes holding the organisms in the light without turning off the photomultiplier power supply. An overall view of the equipment is shown in Figure 17. In the determination of the intensity of luminescent flashes, additional computations, made necessary because of the different distance from the photomultiplier cathode to the working cuvette and luminescence reference standard, considerably complicate data reduction and are a source of error. Consequently, the design was changed and the reference standard housed in a special frame in the space for the working cuvette.

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To study the luminescence of individual organisms under different stimuli, several kinds of cuvettes have been designed (figure 18). The cuvette for chemical stimulation (a) is the simplest. Like the others, it is made out of polished transparent organic glass. By means of a syringe, formalin or alcohol is introduced in different ratios to the volume of water in the cuvette in the apparatus (from 1/50 to 1/2). A disadvantage of this kind of stimulation is that a mechanical excitation of the luminescence occurs when a portion of the stimulant is added. Though in the case of a considerable concentration of added substances this plays no major role, since the organisms die off rapidly and their death is accompanied by luminescence of the highest possible intensity, in a low concentration it is not at all clear whether the flash is caused by mechanical or chemical action. It is also difficult to check the stimulating action on the recorder while simultaneously recording the flash.

For mechanical stimulation, a cuvette (fig. 18,b) has been developed that is distinguished by having a soft rubber diaphragm on its bottom. A light organic-glass plate is connected to the diaphragm of an electromagnetic microphone (type DEM) by means of a flexible steel rod passing through the rubber diaphragm. The microphone is powered by a G3-4 audio oscillator with an adjustable range of frequencies and voltage amplitude. Like the mechanical stimulation system described earlier (Nicol, 1958b), this design has a great time lag. In our studies mechanical stimulation was used to determine the dependence of luminescence intensity on stimulation intensity at different frequencies.

Electrical stimulation was conducted in two kinds of cuvettes. One of them (fig. 18v) resembled in design the cuvette proposed by Nicol. [48] The essential difference is that the somewhat modified form of the agar transition bridges makes it possible to obtain in the working space a field close in form to a plane parallel field. This excludes change in the magnitude of stimulation when the organism is shifted in the working space of the cuvette. Contacts connect the electrodes to the electric pulse generator through the roof of the dark chamber. A shortcoming of the design is the development of electrolysis at the agar-silver electrode junction when current density increases. The second cuvette design (fig.18,g) [49] is simpler. Electrodes made from noncorrosive sheet metal create a plane-parallel field, making it possible to compute current density at different current intensities of the stimulating pulse; experiments were conducted with current intensity in the cuvette ranging from 0.1 to 10mA/mm^2.

Experiments using electrical stimulation have confirmed that this kind of excitation is most satisfactory. Broad regulation limits permit the selection in experiments of the necessary parameters of the stimulating pulses which are recorded with the requisite degree of accuracy. It must be noted that in our practice isolated instances have occurred when the action of the electric pulse during high current density did not cause a flash of light, while the subsequent addition of formalin led to luminescence. This fact can scarcely be explained by a high excitation threshold of light reaction.

For electrical stimulation, an electric pulse generator consisting of circuit units (see fig. 16) has been made. The instrument permits smooth changing the parameters of the stimulating rectangular pulse within the following limits: pulse current intensity from 0.01 to 3A, pulse duration from 1 to 500ms, frequency from 1/30 to 500Hz. In some instances, industrial EI-1 or EI-3 pulsers have been used for the same purposes. These instruments have parameters close to those given above, with the exception of pulse current whose value cannot exceed several tens of mA. Besides rectangular pulses, the instrument can generate triangular and trapezoidal pulses.

As seen from a description of the equipment, not only integral, but spectral light pulse characteristics as well can be studied. The spectral characteristic is determined by successive stimulation of six organisms of one species. Each time the interference light filter in front of one of the photomultipliers is changed. Flashes of individual luminescents differ considerably with respect to intensity, consequently, the readings obtained for each sector of the spectrum separated by a light filter are corrected on the basis of the flash intensity recorded by the integral photomultiplier. Inasmuch as the energy reaching the

multiplier cathode through the narrow-band light filter is considerably less than in integral recording, the signal is sent through an S1-4 d.c. amplifier with a variable amplification factor.

The design of our laboratory equipment and the method of determining the luminescent flash spectrum in many regards are similar to the equipment and methods developed by J. Nicol (1960b) to study the spectral characteristics of fish luminescence. Figure 19 shows the layout of the laboratory equipment. The fish is placed in vessel (6) /50 which is filled with sea water. Flashing of the fish photophores is elicited by mechanical stimulation by means of compression. Light from the object under study in dark chamber (5) strikes semitransparent plate (4) and is separated into two beams. One of them is registered by horizontal photomultiplier (2), while the second passes through a light filter (3) located in one of the openings in the filter disc (7) to the vertical photomultiplier. The vertical and horizontal photomultipliers have maximum sensitivity in the blue part of the spectrum. Signals from the photomultipliers pass through the d.c. amplifiers to the electronic oscillograph. The displacement of the beam on the oscillograph screen is photographed on moving paper. Light filters with a broad pass band are used in the equipment, thereby complicating computations somewhat.

Flash intensity recorded by the vertical photomultiplier with each light filter is corrected on the basis of the integral intensity of the flash received by the horizontal photomultiplier. The spectrum of the luminescent flash is computed in the first approximation from experimental data taking into account the spectral characteristic of the photomultiplier and the spectral transmission curve of each of the light filters. In the second, more refined computation, the spectral distribution of the luminescent flash energy obtained is considered in addition to the spectral characteristics of the detector and light filters. In those rare cases in which the experimenter has a large number of organisms of one species available, a simpler method of determining the spectral composition can be used. This method was /51 used by Nicol (1958b) to determine the radiation spectrum of Noctiluca. A stream of water containing the bioluminescents flows in front of the photomultiplier cathode. During the flow, light flashes of the luminescents occur in the stream. The total duration of luminescence is 3-4 seconds, during which time its intensity increases rapidly and then falls. A disc made of lightproof material along whose edge the light filters are mounted moves between the photomultiplier cathode and the object under study. The rate of disc rotation was somewhat greater than 1 r/s. Two blue-green or green filters are placed in adjacent disc apertures on both sides of each light filter of the basic spectral range. The value obtained from the records for each basic spectral filter is then corrected on the basis of readings from the two auxiliary filters. This makes it possible to take into

account change of the integral intensity of the luminous flux during the measurements and to obtain a curve of the relative spectral distribution of luminescence.

Of great interest, in our opinion, is a somewhat different approach to obtaining relative and absolute spectral characteristics of the light pulses of bioluminescents--the recording of the spectrum of the single flash. Multichannel equipment (5-7 channels) to record the spectral characteristic of the luminescent flash of one organism has been developed in the Laboratory of Photobiology of the Institute of Physics of the Siberian Division of the USSR Academy of Sciences. FEU-29 or EEU-64 photomultipliers (the spectral characteristics of the multipliers are the same) serve as light detectors. Each multiplier records the radiation in a narrow sector of the spectrum separated by an interference light filter, and is calibrated in energy units ($\mu W/cm^2$) from a standard reference source (C^{14}-activated phosphor). The multibeam circuit makes it possible to compare the spectral composition of the radiation of individual bioluminescents of one species. In determining spectral characteristics, especially with the aid of the multibeam circuits, the anisotropic character of the light-energy radiation in space characteristic of some organisms (multiplicity of luminous organs in individual species, emission of luminescent secretion, etc.) must be taken into account.

FLASH CHARACTERISTICS OF INDIVIDUAL ORGANISMS

The light signal of most organisms when stimulated is a well formed pulse with a steep leading edge and a smoother trailing edge. The species characteristic is generally expressed very weakly in the shape of the light pulses. Flashes of different organisms differ mainly in time characteristics. Shorter flashes with short latent periods are characteristic of intracellular luminescence. Extracellular luminescence, occurring as the result of the release of a secretion, usually lasts longer. The form of the signal is independent of the kind of stimulation. /52

Up to now, light flashes under stimulation have been obtained in the laboratory from many marine organisms, including the ctenophore Mnemiopsis leidyi (Chang, 1954), in helminths (Nicol, 1957 a, b, c; 1958 d; Johnson, F., Johnson, N., 1959), in the mollusc Pholas dactylus (Nicol, 1958e), in Noctiluca miliaris (Nicol, 1958 b), in the coelenterate Renilla (Cormier, 1960), in Copepoda (David, Conover, 1961; Clarke, Conover, et al., 1962), and in many others. For other marine organisms studied in the laboratory, only the basic parameters of luminescence will be indicated.

PROTOZOA

Radiolaria--The luminescence of Radiolaria, as in testaceous flagellates, can easily be caused by simply shaking the vessel containing the plankton sample. However, it is very difficult to record light signals of individual organisms because of their microscopic size. The duration of the light emissions of the radiolarians Cytocladus major and Aulosphaera triodon under mechanical stimulation is relatively great--up to 2 seconds. Flash intensity is low--only $5.3 \cdot 10^{5}$ μW of light energy falls on 1 cm^2 of multiplier photocathode surface at a distance of 1 cm from the object (Nicol, 1958a, 1960a). It is very difficult to obtain flashes from Radiolaria under electrical and chemical stimulation.

Noctiluca miliaris--Noctiluca responds with flashes to mechanical, chemical, and electrical stimulation. In response to a single electrical pulse, Noctiluca produces a flash with a steep front and smooth trailing edge. Immediately after reaching maximum flash intensity, fading sets in. The light pulse will usually appear 10ms after stimulation and lasts about 100 μs. Flash intensity at a distance of 1 cm from the multiplier photocathode is about $0.1 \cdot 10^{-6}$ $\mu W/cm^2$ of photocathode surface.

Flash amplitude increases with increased strength of stimulation (Nicol, 1958b).

Our measurements of the luminescence of Noctiluca miliaris were carried out by means of the equipment described above. In the case of stimulation with electrical pulses having a frequency from 0.1 to 3 Hz and duration of 4.4, 12.22, and 46 ms (current density in the cuvette was about 1 mA/mm^2), flash duration varied within broad limits from 50 to 752 ms. Flashes lasting from 100 to 250 ms (threshold current density about 0.6 mA/mm^2) predominated. The flash duration of Noctiluca does not depend on the frequency and duration of the stimulating pulses nor on the current density in the cuvette. Even the same organism produced flashes of different duration under two successive identical stimulations. In the case of repeated electric-current stimulations, flash duration does not depend on the number and succession of stimulations (fig. 20). A definite relationship is sometimes observed to exist between flash amplitude and duration; the brighter flashes may last longer than the weaker ones but in some cases the longest flashes are the least bright. Noctiluca flash duration is made up of the time to reach maximum luminescence and the decay time. The time during which luminescence intensity reaches maximum ranges from 12.5 to 50 ms. The time to reach maximum does not correlate with current density in the cuvette, frequency, or duration of the stimulating pulses. This characteristic of light pulses, as well as the decay time, is independent of the total flash duration. The relationship of decay time to total light-pulse duration does not remain constant even for successive flashes of the same Noctiluca. Luminescence decay time is also independent of

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[54]

current density, duration, and frequency of the stimulating pulses. An interesting relationship has been established between the latent period and the duration of the stimulating pulses. As the duration of stimulus action increases, the number of flashes with a protracted latent period increases (Table 1).

TABLE 1. Dependence of latent period of flash of Noctiluca miliaris on the duration of stimulating pulses (occurrence in %)

Stimulating pulse duration, ms	Latent period, ms												
	6.5	7	10	17	34.5	42	47	49.5	59.5	70	88.5	96	120
4.4	84.4	1.9	9.8	3.9	0	0	0	0	0	0	0	0	0
22	0	0	0	0	9.8	1.7	75.3	6.6	3.3	3.3	0	0	0
46	0	0	0	0	0	0	0	4.9	1.2	81.6	3.7	4.9	3.7

NOTE: Current density and stimulation frequency are the same; latent period is calculated from the moment stimulation begins.

The statistical distribution of flashes of different Noctiluca based on amplitude classes does not show any dependence of luminescence intensity on the frequency and duration of stimulating pulses nor on current density above threshold values. This fact is very interesting, since it attests the independence of flash intensity from the character of the stimulus.

In the case of chemical stimulation (formalin, ethyl alcohol, ammonia solution in concentrations from 0.8 to 25%), individual Noctiluca flashes have the same form and time characteristics as in the case of electrical stimulation. However, in chemical stimulation, the luminescence has certain peculiarities. First, in chemical stimulation, it is difficult to obtain several successive flashes from a single organism. Usually, the Noctiluca produces a single light pulse under chemical stimulation. Subsequent stimulations are ineffective. We succeeded in recording two flashes in response to two successive stimulations; (there was no third flash) only when 4% ethyl alcohol was used in stimulation. The duration of individual Noctiluca flash under chemical stimulation depends on the concentration of chemical stimulus (stimulus strength): As the concentration increases, the light-pulse duration increases. Complex flashes with many peaks and having a duration of up to several seconds will occur (fig. 21). [55] Subsequent increase in stimulus strength results in the disappearance of pulse luminescence and the appearance of continuous luminescence. Usually, when such luminescence occurs, the cell dies (Gitel'zon, Chumakova, 1966).

Little is known about the systems responsible for controlling luminescence in Noctiluca. There are only data about the definite role of the SH-group of proteins (most likely, some kind of ensyme systems) in the formation of the light pulse. Flashes caused by mechanical stimulation easily change to nonpulse luminescence on the addition of cadmium chloride, which, as is known, blocks the SH-group (Koshtoyants, 1953). Small concentrations of alcohol, formalin, and iodine do not produce this effect.

* * * * *

Pyrocystis pseudonoctiluca--Luminescence of this flagellate is most easily provoked by mechanical stimulation; chemical and especially electrical stimulation are less effective. Luminescence of P. pseudonoctiluca under chemical stimulation (formalin and ethyl alcohol) was recorded by V. N. Greze and colleagues in 1966. Flash duration varied from 125 to 500 ms and showed a direct dependence on the concentration of the reagent added (up to a specific concentration). Another flagellate, Gonyaulax polyedra, under chemical stimulation produces flashes lasting 130 ms (Hastings, Sweeney, 1957b). /57

Flashes of single P. pseudonoctiluca under electrical stimulation were observed by R. I. Chumakova, R. N. Utyushev, and I. I. Smol'yanov in 1967. A luminescent signal appeared only at a stimulating-pulse frequency of 1 Hz; pulse duration was 50 ms and current density in the cuvette was about 6 mA/mm^2. The flashes were separate, single-peaked, and exhibited different characteristics even when the same organism was under repeated stimulation. Their duration varied from 100 to 690 ms; most were flashes lasting 200 ms. Flash energy was from $7.5 \cdot 10^{-4}$ to $6.3 \cdot 10^{-3}$ $\mu W/cm$ at a distance of 5 cm from the multiplier photocathode. The overwhelming number of pulses had an intensity of about $3 \cdot 10^{-3}$ $\mu W/cm^3$. A peculiar luminescence buildup appeared. During repeated stimulation, the flash amplitude increases, reaches a maximum and then begins to fall off (fig. 22,a). Greatest flash brightness was observed after 20 stimulations. Some correspondence was found to exist between the amplitude and duration of each flash (fig. 22, a, b). /58

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CRUSTACEA

Copepods--V. N. Greze and colleagues in 1966 reported the luminescence of 12 species of Copepods in the Caribbean Sea of which eight were identified as luminescent for the first time. Figure 24 shows the luminescent signals of some of these crustaceans. /60

Under chemical stimulation (alcohol and formalin in various concentrations) the flash duration of Pleuromamma gracilis varied from 10 to 125 ms, Heterorhabdus spinifrons from 7 to 700 ms, the pulse of Scolecithrix danae lasted up to 2 seconds. We recorded flashes of Oncaea sp., Pleuromamma sp., Metridia sp., Euchaeta sp., and Oithona sp. under electrical stimulation. The light responses of these organisms to the first electrical pulses were usually complex, lasting up to 20 seconds. Subsequent flashes were simple and shorter, from 1 to 5 seconds (fig. 25). In Oncaea and Pleuromamma a dependence of flash duration on the duration of the stimulating pulse was noted: the longer the stimulation, the greater the flash duration. Moreover, /61

Duration of Stimulation m/sec	Flash duration, sec.	
	Pleuromamma	Oncaea sp.
5	1.5	1
10	2.4	1.5
50	3.8	--
100	9.2*	3.6*

*Flashes with six peaks

the number of peaks of a complex flash increases with longer stimulating pulses. When more protracted pulses were used in stimulation, the level of continuous luminescence increased. Apparently, this was caused by the retention of luminous secretion in the ducts of the luminescent glands.

An entirely different relationship between flash duration and amplitude in successive stimulation was found to exist in the Copepods than is the case in luminescent Flagellata (fig. 26). Flash amplitude decreases steadily, while the duration, in cases of successive stimulation, sometimes increases. Possibly, the increase in flash duration occurs because of a decrease in the rate of discharge of the luminescent secretion from the glands. The drop in luminescence intensity in cases of protracted stimulation is apparently explained by a decrease in the number of active granules in the glands.

Copepods are distinguished by their high luminescence intensity, which considerably exceeds that of the Protozoa. Metridia longa, M. lucens, and Pleuromamma robusta produce flashes with intensities from $0.0045 \cdot 10^{-2}$ to $2.015 \cdot 10^{-2}$ $\mu W/cm^2$ at a distance of 1 cm from the photomultiplier photocathode (Clarke et al., 1962). The data we have obtained on the flash intensity of Copepods is given below.

Species	Flare energy, $\mu W/cm^2$ at a distance of 5cm from the photocathode
Pleuromamma sp.	$1.2 \cdot 10^{-3} - 7.6 \cdot 10^{-2}$
Metridia sp.	$3.7 \cdot 10^{-3} - 2.5 \cdot 10^{-2}$
Euchaeta sp.	$9.4 \cdot 10^{-3} - 4.9 \cdot 10^{-2}$
Oithona sp.	$4.2 \cdot 10^{-2} - 4.6 \cdot 10^{-2}$
Oncaea sp.	$2.2 \cdot 10^{-3} - 3.3 \cdot 10^{-2}$

Other Crustaceans--Very little is known about the luminescence characteristics of other crustaceans. Under electrical stimulation flash duration in Ostracoda varies from 0.01 to 54.5 seconds, while the latent period has a duration of from 0.01 to 5 seconds (Rudyakov, 1967a). With respect to brightness the flashes resemble those of Copepoda. On the basis of data obtained in the Laboratory of Photobiology of the Institute of Physics, the energy of luminescent signals of Ostracoda has a value of from $5.3 \cdot 10^{-3}$ to $6.3 \cdot 10^{-2}$ $\mu W/cm^2$ at a distance of 5 cm from the photomultiplier photocathode. /62

Again, little is known about the luminescence characteristics of the euphausiids. A flash of Euphausia pacifica lasts about 30 seconds, (Nicol, 1958a), and a figure of 22 seconds has been given for the light-pulse duration of Meganyctiphanes norvegica (Clarke, Conover, et al, 1962). The flash intensity of Euphausia pacifica reaches $2 \cdot 10^{-3}$ $\mu W/cm^2$ at a distance of 1 cm from the photomultiplier photocathode (Kampa, Boden, 1957).

Data on the flash duration and luminescence intensity of other marine organisms under stimulation are given in Table 2. We also recorded luminescence in the deep-sea fish Astronesthes niger (V. E. Becker's determination) under mechanical and chemical stimulations (fig. 27).

TABLE 2. Flash duration and intensity of some marine bioluminescents. /64

Organism	Flash duration, sec.	Intensity at Distance of 1 cm, $\mu W/cm^2$	Temperature °C	Reference
Coelenterata				
Atolla wyvillei	1.6	$2 \cdot 10^{-3}$		Nicol, 1958a
Vogtia spinosa	11.2	$3 \cdot 10^{-3}$	17-19	
Aeginura grimaldii	2-6	$0.5 \cdot 10^{-5}$ $1.3 \cdot 10^{-5}$	15	Clarke, Conover, 1962
Periphylla periphylla	0.8-4	$0.13 \cdot 10^{-5}$ $0.3 \cdot 10^{-3}$	15	Same
Aequorea forskalea	0.2-0.4	--	14-16	Davenport, Nicol, 1955
Pennatula phosphorea	1.44	$7 \cdot 10^{-6}$	--	Nicol, 1960a
Ctenophora				
Mnemiopsis leidyi	0.3	--	21-23.5	Chang, 1954
Beroe ovata	0.16	--	--	Nicol, 1958a
Annelides				
Acholoë astericola	0.08	$1.1 \cdot 10^{-4}$	16-18	Nicol, 1958a
Chaetopterus variopedatus	5-10	--	--	Nicol, 1958a
Crustacea				
Acanthephyra pelagia	3-4	$11.2 \cdot 10^{-4}$	15	Clarke, Conover et al 1962
Tunicata				
Pyrosoma atlantica	7.2-30	$4 \cdot 10^{-2}$	25	Nicol, 1958a
Teleostei				
Searsia koefoedi	--	$2.8 \cdot 10^{-2}$	21-24	Nicol, 1958a
Myctophum affine	1.3-4	$0.1 \cdot 10^{-5}$	23	Clarke, Conover 1962

NOTE: For Aeginura grimaldii, Periphylla periphylla, and Acanthephyra pelagia, the distance was 15 cm; for Myctophum affine it is 14 cm.

* * * * *

PHYSIOLOGY AND BIOCHEMISTRY OF MARINE LUMINOUS BACTERIA

/72

The luminous bacteria cultivated in the Laboratory of Photobiology of the Institute of Physics, Siberian Branch, USSR Academy of Sciences, which belong to the genus Photobacterium, are bacilli, single or paired, 0.5-2 micron in size, Gram-negative.

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/74

Among the luminous bacteria of the genus Photobacterium, isolated during the 38th expedition of the VITYAZ, were strains that grew well at temperatures of 30°, 25°, 12°, 10°, and 6°. Figure 31 presents a curve showing the dependence of the rate of growth of one of the strains (no. 5) on temperature, obtained in our laboratory by S. Ye. Preobrazhenskaya. In some species of luminous bacteria, the temperature optima for growth and luminescence coincide (Photobacterium fischeri, Vibrio luminosus, V. indicus). In other species (Photobacterium harveyi, Ph. phosphoreum, Vibrio albensis), the temperature optimum for luminescence is lower than the growth optimum: in Ph. harveyi, 28°; in Ph. phosphoreum, 20°; and in V. albensis, 22° (Brown, Johnson, Marsland, 1942). For Bacterium issatchenkoi, the temperature optimum of luminescence was found at 20°. Pacific Ocean strains of photobacteria display the greatest luminescence intensity at temperatures of from 25° to 35° (fig. 31).

The luminescence of marine photobacteria, like that of most marine organisms, is in the blue-green region of the spectrum. Figure 32 gives the luminescence spectrum of Ph. fischeri (Strehler, Cormier, 1953), and Table 3 lists the spectral distribution of the radiation of other species of bacteria. The luminescence spectrum of Bacterium issatchenkoi (with a correction for the spectral sensitivity of the photomultiplier photocathode), on the basis of our data, is an asymmetrical curve stretching from 420 to 540 mμ with a maximum at 478 mμ. The luminescence spectrum of Pacific Ocean bacteria has a maximum at 483 mμ.

TABLE 3. Spectral composition of radiation of luminous bacteria.

Species	Spectral range, mμ	Maximum mμ	Reference
Photobacterium phosphoreum	420-600	472	Spruit, van der Burg, 1950
Photobacterium splendidum	410-650	489	Same
Photobacterium fischeri	430-600	496	Same
Photobacterium fischeri	420-600	500	Harvey, Chase, McElroy, 1957
Bacterium Issatchenkoi	420-600	475	Voytov, Yegorova, Tarasov, 1960

The bioluminescence of bacteria is closely related to their metabolism. This is attested to by the following facts: (1) only live cells luminesce, (2) the luminescence of intact bacteria under optimum conditions occurs without any stimulation continuously for a long time. /76

Investigation of the relationship between luminescence and the phase of development of the bacterial culture has shown that the curve of luminescence intensity of the bacteria does not correspond to the curve of culture growth. Thus, during the period of lag phase, luminescence intensity increases, while the growth of the culture is slowed. In the logarithmic growth phase, the bacterial luminescence intensity increases more rapidly than the number of cells. The relationship between luminescence and cell development can be traced most completely in a synchronized culture. The fact that in synchronized cultures there is a separation in the time between growth and cell division permits a study of culture development by phases repeating those of the development of one cell. Synchronization of division of luminous bacteria in our study was achieved by a sharp temperature change. It was established that in the case of a synchronously dividing culture, the luminescence curve does not coincide with the curve of culture growth (fig. 33). While the increment in numbers proceeds in stages, interrupted by an interphase, the luminescence intensity increases continuously. Computations have shown that increase in luminescence intensity occurs solely because of the growth of aggregate cell surface. This attests that the radiation sources in a bacterial cell are multiple (Chumakova, 1963, 1965).

Bacterial bioluminescence is an oxidative and aerobic process. It is therefore of interest to study the relationship between luminescence and respiration. Research results show that for all luminous species studied (Bacterium issatchenkoi, Photobacterium sp.--Pacific Ocean strains), the temperature optima of luminescence and respiration do not coincide. The optimum oxygen requirement is always higher than the luminescence optimum, i.e., the temperature coefficients of these two processes differ.

Investigation of the relationship between bacterial luminescence and the activity of dehydrogenases oxidizing glucose, lactic acid, and components of the citric acid cycle (succinic, fumaric, and citric acid) has shown that there is a direct relationship between luminescence intensity and the rate of dehydrogenation of substrates at the same temperature. Luminescence intensity and, consequently, the rate of oxidation of the light-emitting substrate, is higher, the more rapidly the energetic substrate is dehydrogenated. A direct relationship was also found between luminescence and oxygen requirement. The more energy liberated in the oxidation of nutrients, the more intense the luminescence (Chumakova, Yegorova, 1964).

The question of the quantitative relationship between luminescence and respiration is also interesting, i.e., the amount of energy expended in luminescence. To explain this, the quantitative relationship between the luminescence of bacteria and their need for glucose--the energetic substrate whose addition to a suspension of washed bacteria is accompanied by the greatest increase in luminescence intensity (compared with organic acids) was investigated. The increase in luminescence intensity after adding glucose has a clearly defined maximum, after which a gradual return to the initial level of luminescence occurs. By means of an installation calibrated according to a certified brightness standard, the total radiant flux from a suspension of luminous bacteria was measured. After having determined the radiant flux before and after the addition of glucose and the curve of variation of the radiant flux from the moment the glucose was added to the maximum, we computed the light energy liberated by the bacteria during the period from the addition of the glucose to luminescence maximum. The amount of glucose required by the bacteria during this time was determined by the anthrone method. Measurements and calculations showed that $1 \cdot 10^{-4}$% of the total energy released in the oxidation of the glucose is expended on luminescence (Gitel'zon, et al., 1965a). Thus, the quite noticeable external effect of vital processes--bioluminescence--requires very little energy expenditure from the cell. /77

The constant relationship between the emitted energy and that liberated in dehydrogenation permits us to regard bacterial bioluminescence as a means whereby the cell eliminates energy (comprising a constant amount in the total balance) liberated by glycolysis. This energy, for a still unknown reason, cannot be utilized. Bioluminescence is a direct energy loss from the oxidation channel. Thus, bacterial luminescence is a respiratory phenomenon, although luminescence is less sensitive to reduce oxygen pressure than is respiration. There are limits within which bacterial luminescence does not depend on oxygen concentration. According to data obtained by I. I. Gitel'zon et al. (1967), the luminescence intensity of a continuous culture of luminous bacteria does not depend on the concentration of dissolved oxygen until it drops to a value of 0.22-0.36 ml/l (with continuous agitation).

The concept of the continuous, nonpulse nature of bacterial luminescence is widespread (Harvey, 1952). However, the continuity of luminescence of many millions of bacteria (usually the luminescence of such number of cells is observed) may not reflect the true nature of cell luminescence. In this regard, investigations of the process of bacterial luminescence have been undertaken using photomultipliers having a single-electron noise-pulse spectrum (Fish, et al., 1967). The integral and differential amplitude distribution of noise pulses and signal pulses was obtained from a small number of bacteria. Analysis of the spectral distribution of /78

the noise and signal pulses showed that there are no light pulses lasting less than 1 μs in bacterial luminescence.

The lability of the bioluminescent apparatus of the bacteria permits studies of the level of bacterial biosynthesis and, what is very important, at different level variations, with minimum time lag from the change in luminescence intensity. In this regard, it was interesting to record separately the final effect of metabolism in the form of an increase in the bacterial biomass and its intermediate stages in the form of bioluminescence under conditions of continuous culture with stabilization of the basic parameters determining the level of metabolism. Continuous culture of bacteria was accomplished with a specially designed installation, consisting essentially of a closed circuit with thermostatically controlled jacket and sensors of recorded parameters--culture density, temperature, luminescence, and concentration of oxygen dissolved in the suspension (fig. 34) (Gitel'zon, et al., 1965b; Gitel'zon, et al., 1967). In continuous culture, luminous bacteria can metabolize and luminesce normally for a long period without signs of culture degeneration. The same dependence of growth and luminescence rate on temperature was found for a continuous culture as for a cumulative culture. The essential difference is observed in the time of a generation in the cumulative and continuous cultures. At the optimum temperature (30°) for the growth of strain no. 2 (Photobacterium sp.), the time of one generation in cumulative culture is 40-50 minutes; in continuous culture of the same strain, it decreases to 8-10 minutes.

Under conditions of continuous culture, as in the case of a synchronized culture, the luminescence intensity growth outstrips the biomass growth. Thus, in the period of culture adaptation when the growth rate increased five times, luminescence increased 50-100 times (Gitel'zon, et al., 1965b; Gitel'zon, et al, 1967).

According to Harvey's data (1952), the quantum yield of bacterial luminescence in relation to required oxygen is from 1/100 to 1/1000 (i.e., from 100 to 1,000 O_2 molecules are required for 1 light quantum). The use of an oxygen-concentration sensor in the installation for continuous culture of luminous bacteria permits the rate of oxygen requirement to be determined. It was found that at 25° an average of $8.33 \cdot 10^{-2}$ μl of O_2 are required per hour for one million cells. According to A. M. Fish's computations, an average of 62 oxygen molecules are required for 1 light quantum emitted by bacteria.

* * * * *

Crystalline luciferase was obtained from luminous bacteria of the genus Photobacterium cultivated in the Laboratory of Photobiology of the Institute of Physics by a group of Leningrad scientists headed by S. I. Borkhsenius (1967). Fractionation of bacterial extract with $(NH_4)_2SO_4$ yielded a fraction containing 20% of the total amount of protein in the extract and 80% of the total luciferase and NAD-H_2-oxyreductase activity. In a chromatographic column with DEAE-cellulose, this material was divided into two fractions. One was characterized by chiefly luciferase activity (luminescence in the presence of reduced FMN), the other by NAD-H_2-oxyredactase activity. The mixture of these fractions, in addition, possessed luciferase activity in the presence of oxidized FMN. When these fractions were crystallized, in the case of the luciferase, yellow-green crystals in the form of hexagonal prisms (with eight faces) were obtained, and in the case of NAD-H_2-oxyreductase, colorless crystals in the form of convex parallelohedra with 12 faces were obtained (fig. 35). /80

INSTRUMENTS FOR INVESTIGATING BIOLUMINESCENCE IN THE SEA

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In measurements of bioluminescence the Institute of Biology of the Southern Seas of the UkrSSR Academy of Sciences (Bityukov, et al, 1967) uses a bathyphotometer equipped with exciter lattice having a light lock* attached at a distance of 20 cm from the photodetector /86

*The device consists of two plates with apertures separated from each other in such a way that the apertures of one plate did not overlap those of the second plate.

to limit the instrument's field of view. This design substantially reduces the light background on the photodetector from the astronomical component and improves somewhat the conditions for registering excited bioluminescence. However, the excitation level passed by the lattice (and the instrument itself), as before, depends on sea state, ship motions, etc., and is not controlled.

An original installation to study excited bioluminescence was employed by G. S. Karabashev. To measure excited bioluminescence in an open volume (luminescence was excited by rotating a 60-cm diameter wheel at a speed of 40 r/min), a filter-photometer, mounted on an underwater photometric bench, developed by the author (Karabashev, 1967), was used that permitted changes to be made in the distance between exciter and detector. With this installation, the mean spectral energy distribution of bioluminescence was measured directly in the sea for the first time.

These measurements permitted some inferences to be drawn on the geometry of the field of excited bioluminescence and on its lifetime. Among the shortcomings of the method, are the relatively low and non-uniform excitation level and the possibility of uncontrolled selectivity of bioluminescence excitation of the various plankters because of their varying mobility.

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ELECTRICAL CIRCUITS

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The possibility of measuring low-intensity radiant fluxes by means of photomultipliers (FEU) made possible relatively simple, reliable photometers with the sensitivity necessary to detect the luminescence of marine organisms at any depth.

Highly sensitive photomultipliers with maximum sensitivity in the visible part of the spectrum are used as photodetectors in bathyphotomers. In non-Soviet designs, nine-stage type 931A photomultiplier for ten-stage type 5819 instruments are most often used. /89
Among Soviet designs, an FEU-29 thirteen-stage photomultiplier with electrostatic focusing (or the similar FEU-19A) is usually used with an anode sensitivity up to 1,000 A/lm and relative spectral sensitivity of the photocalthede of 300-600 nm. In some designs, an FEU-15 twelve-stage louver photomultiplier with a spectral sensitivity range of 300-750 nm and spectral sensitivity maximum near 480 nm was used in order to extend the spectral sensitivity in the direction of the longer wavelengths. The sensitivity of this photomultiplier is about an order lower than that of the FEU-29.

On the whole, it can be noted that the assortment of photomultipliers put out by industry is sufficiently broad and that there is no particular difficulty in selecting a photomultiplier to measure bioluminescence in situ.

Different ways are used to deliver supply voltage to the photomultiplier. For example, in G. S. Karabashev's instrument (Karabashev, 1967) high voltage is delivered directly from shipboard. In some instruments developed in the Institute of Physics of the Siberian Division of the USSR Academy of Sciences, power supply is delivered from a shipboard low-voltage source via a high-voltage converter located inside the housing of the submerged sensor. Self-contained power-supply systems are also used. Figure 40 shows the schematic of an instrument in which the power supply is a low-voltage source inside the housing of the submerged sensor.

* * * * *

An example of a unit with self-contained power supply in which the photomultiplier operates in a linear mode is the bathyphotometer shown in Figure 40, developed in the Institute of Physics of the Siberain Division of the USSR Academy of Sciences (Filimonov, et al, 1965). The unit is supplied by 10 type STs-5 silversinc batteries (connected into the circuit through a stabilizer based on semiconductor triodes P_1, P_2, P_3 of type P-15 and P-201). The power so obtained is sufficient to operate the unit continuously for 10-12 hrs. The large power reserve considerably simplifies the care and maintenance of the instruments on expeditions. The low voltage is stepped up to 1,200-1,700V by means of the converter formed by triodes P_4, P_5 (type P-201) and six D-211 diodes and delivered to the FEU-29 photomultiplier. /91

The instrument is switches on and off by high-voltage (200V) pulses delivered from shipboard by means of a circuit formed by two stabilitrons and two relays. Use of this circuit permits KOBD-4 single-conductor logging cable to be used for communication with the instrument. A major shortcoming of this instrument circuit is that it is necessary to have some idea about the signal values likely to be encountered in the depth interval of interest at the station being occupied, so that the instrument sensitivity necessary for successful measurements can be established. In this design, the sensitivity can be regulated by changing the voltage taken from the secondary winding of the converter transformer.

When a cable with a large number of conductors is used (for example, the KTB-6 3-conductor cable), the circuit can be powered from shipboard. In this case, the stabilizer and remote control units are removed. This variant of the circuit is convenient to use, since instrument sensitivity can be regulated (by varying the low voltage delivered from shipboard) within a broad range during actual operation, being guided by the best conditions for signal recording.

The extraordinary simplicity of the submerged sensor in this circuit variant makes the instrument quite reliable in use. One of the variants of the internal arrangement of the sensor is shown in Figure 41.

Compactness is another positive feature of the sensor. Figure 42 gives an external view of a portable bathyphotometer set having this type of electrical circuit. This instrument is intended for use from small vessels and craft to study bioluminescence in shallow waters, for example, in lagoons. The sensor of this instrument can also be carried by a skin diver or aqualunger. /93

In the linear mode of the photomultiplier, the instrument has a relatively narrow dynamic range, of the order of 40 dB. The dynamic range can be substantially widened by using the photomultiplier in the logarithmic mode.

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There are also bathyphotometer circuits in which the photomultiplier can operate in both the linear and logarithmic modes (fig. 43). Figure 44 shows the schematic diagram of the measurement unit of the submerged sensor of the bathyphotometer developed in the Institute of Physics of the Siberian Division of the USSR Academy of Sciences. The FEU-15 serves as photodetector in this bathyphotometer. Depending on the mode selected (linear or logarithmic), either an L_1 tube (type 6E5P) or L_2 tube (type 6Zh11P) is connected to the photomultiplier. Tube L_2 does not operate in the logarithmic mode and is plugged into the middle panel, closing contacts 3-1. Voltage generated by the FEU anode current on resistor R_{24} is delivered to the control grid of tube L_1. Voltage from the divider (proportional to the logarithm of the luminous flux) is sent to the emitter follower based on silicon triodes PP1 and PP2 to match the output impedance to the loop oscillograph and then passes through limit resistor R_3 to the cable connecting the submerged part to shipboard. 95

In the linear mode, tube L_1 is plugged into the middle panel and bridges contacts 1-6 and 1-9, i.e., the high-voltage source plus is connected to the photomultiplier anode. The signal from the photomultiplier anode goes to the grid of tube L_2, connected as a cathode follower, and then passes through resistor R_{31} to the cable. Toggle switch T_1 permits the cathode follower to be disconnected and the photomultiplier anode to be connected to the connecting cable, thereby ensuring a record of the signal on the loop oscillograph directly from the photomultiplier anode.

Electric motor M rotates the disc placed in front of the photomultiplier cathode on which is attached a standard reference light source (C^{14}-activated phosphor), thereby ensuring the reception of two calibrating signal levels ("dark" and "standard"). The electric motor is started either once a minute automatically or on command from shipboard. An overall view of the submerged part of this bathyphotometer (without housing) is shown in Figure 45.

To complete the survey of bathyphotometer electric circuits, mention should be made of the one used in the Hardy-Kay instrument (1964) in which mechanical modulation (modulation frequency 200 Hz) of the luminous flux reaching the photocathode is used to reduce the effect of dark current. The photomultiplier in the instrument operates in linear mode; sensitivity is changed by varying the photomultiplier anode voltage by means of resistors. The modulated signal

from the photomultiplier output is then amplified by a three-stage amplifier (using EF86 and ECL82 tubes). After amplification, the signal is detected and sent onboard by cable. The instrument has a sensitivity of 10^{-3} $\mu W/cm^2$, considerably inferior to instruments using other circuits, and can record only those bioluminescent flashes that last longer than 0.5-1 second. Thus, the true picture of bioluminescence is seriously distorted. [96]

Use of this circuit, in our opinion, is scarcely worthwhile, chiefly because of the unjustifiably complicated design and instrument circuit. The level of circuit noise, which is reduced by introducing modulation of the useful signal, by proper selection of circuit elements, and are selected especially by selection of a sensitive photomultiplier with a low dark-current level does not limit bathyphotometer sensitivity. The small signal-strength amplification that is required in some instances can be entirely provided for by simpler means, for example, by means of an emitter or cathode follower. To satisfactorily reproduce the forms of bioluminescent pulses, the modulation frequency must be increased by not less than 10-20 times.

Thus, to measure bioluminescence signals in situ, bathyphotometer circuits can be used in which photomultipliers may operate in logarithmic or linear mode. Selection of one or the other mode is determined by the particular purpose of the bathyphotometer and the conditions of use. It would be very desirable to use in the instrument devices that would permit its sensitivity to be calibrated during the actual measurements. Instrument sensitivity is preferably changed by switching neutral attenuators. To improve the linearity of the logarithmic characteristic in the operating intensity interval of detected luminous flux in instruments with a logarithmic photomultiplier mode, it is also expedient to use neutral attenuators that change the photometer's sensitivity range.

RECORDING SYSTEMS

Recording bioluminescence in situ is quite a complex matter. The duration of bioluminescent flashes of individual organisms varies within rather wide limits--from a tenth of a millisecond to seconds; the amplitude range of these flashes also comprises several orders. The average number of bioluminescence pulses recorded by an instrument in a minute can vary at different depths and in different regions from units to, roughly, thousands. In one horizon, the number of flashes during different minute intervals can change several times. Moreover, the photodetector usually also records light of astronomical origin propagated in the water; the level of this light does not remain constant. [97]

Computations show that in a statistically representative sample of bioluminescence signals at one horizon (measurement time at one horizon is three minutes) there can be on the order of $>.10^4$ different amplitude values of the signal recorded by the instrument. Of course, it would be unrealistic to expect complete reduction of the total volume of incoming information by any but automated means. On the other hand, use of automatic information processing assumes an automatic presentation of the data obtained in a form suitable for such processing. From this point of view, the recording systems widely used at present in measuring bioluminescence in the sea are unsatisfactory.

CRT oscillographs with signal recording on photographic paper*

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*A bioluminescence record in situ so obtained is usually called a bathyphotogram.

(in Soviet designs, N700 or N008 loop oscillographs are usually used) have been most widely used to record bioluminescence signals. Use of a photorecord with proper selection of loop galvanometer ensures that a record completely suitable for the integral light signal incident on the photodetector will be obtained.

An inconvenience of this recording method is the need for subsequent development of the photorecord and the difficulty of exerting quality control over the record right in the measurement process. Usually visual control of incoming information is provided for by a low-frequency cathode-ray oscillograph, for example type S1-4 or S1-19.

A shortcoming of this recording method is the great amount of labor involved in processing the bathyphotograms, as the computation of results from the bathyphotograms scarcely lends itself to automation. Therefore, the literally kilometers of bathyphotograms, obtained during an expedition, receive mostly only preliminary, rather tentative processing, while only a negligible part of the records is subjected to analysis. Moreover, the selection of material for detailed analysis is often subjectively determined by the investigator.

Considering the practical impossibility of completely processing all bathygrams, it is often necessary to use recording systems in which a considerable part of the information is unavoidably lost in order to present the basic mass of material in a more compact form. In some cases, the selection of such recording systems, which are not completely appropriate for the signal measured, is determined by special design features of the instrument or by other circumstances. /98

Among similar limitations of bioluminescent signal recording systems is, for example, the computation of the number of bioluminescence pulses during measurement in a particular horizon. Such a recording system could be provided in the instrument design as either a main or auxiliary system. For example, during the 17th expedition of the LOMONOSOV (Artemkin, et al., 1965), bioluminescence pulses at some stations were counted by means of the PS-10000 scaler. Pulse count was accomplished as a single recording system in the instrument to measure excited bioluminescence described by Backus et al. in 1961, in which the signal from the photomultiplier is formed by a delayed multivibrator and then recorded by a pulse counter with a printer.

In counting the number of pulses, the level of the "constant" signal components (due to the astronomical background and the merging of a great number of low-amplitude pulses), the pulse form, and the pulse amplitude distribution are obviously lost. Some idea of pulse amplitude distribution could be obtained by introducing pulse discrimination on a few levels and by counting the number of pulses for each discrimination level.

In many cases, pen recorders whose time-lag exceeds by a considerable margin the duration of the separate bioluminescence flashes are used to record bioluminescence (Clarke, Wertheim, 1956; Neshyba, 1967; et al.). When bioluminescence is recorded at a relatively low value of mean bioluminescent-flash recurrence frequency, recording bioluminescence in situ by means of pen recorders substantially distorts the true picture of bioluminescence in the sea.

Depending on the type of recorder used in this case, two alternatives are possible. If the recorder time lag can be characterized by a time constant, then the recorded signal is unequivocally associated with the input signal and the original signal can be recovered from the graph obtained. A circuit with a large time constant can also be used for signal integration, i.e., to obtain the value of radiant energy incident on the photodetector during a particular time interval.

In many cases, the recorder time lag cannot be characterized by a definite time constant. A typical example is the EPP-09 or EPPV-60 electronic potentiometers in which the time lag is determined by the time for the carriage to traverse the entire scale and the working speed of the reversible motor is not proportional to the unbalance-signal value. In this case, if the rate of change of a signal being detected is notably greater than the carriage speed, then when signals are recorded whose amplitudes are distributed randomly in time, the input signal value has an ambiguous relationship with its recorded value and the distortion of the signal in recording is irreversible. [99]

However, in some cases even that kind of recorder could be used to record bioluminescence signals in situ. This, for example, is the case when recording bioluminescence excited in a large volume at a high excitation level and with a relatively high concentration of luminous organisms in the excited volume. In this case, a precise reproduction of the form of the envelope of the bioluminescent signal has no special sense; of interest is the mean level of excited bioluminescence and changes in this level during a time period not less than a second. Thus, for example, when measuring excited bioluminescence in an open volume, G. S. Karabashev (Karabashev in press) successfully used bioluminescence signal records made on the EPP-09 recorder with a carriage traverse time of 1 second.

Quite promising is the use of magnetic recording media to record bioluminescence signals in situ. Boden et al. (1965) used a tape recorder in an apparatus to study spontaneous bioluminescence. Its design permitted signals from two bathyphotometers to be recorded on one tape track. One channel was recorded by frequency modulation, the second by amplitude modulation. Then the signals from the magnetic tape--slowed by a factor of eight--were rerecorded on a conventional recorder.

A quite universal system for recording bioluminescence signals has been developed and is being used by the Institute of Physics of the Siberian Division of the USSR Academy of Sciences. One variant of this system permits signals to be recorded simultaneously from several bathyphotometers by means of a loop oscillograph. Moreover, the system permits a visual check (on the S1-4 oscillograph screen) of the signal arriving from the bathyphotometers, signal integration (and recording the integrated signal on the EPPV-60 recorder), and a bioluminescence pulse number count for three discrimation levels or three separate recording channels.

Thus, from the discussion above, it is clear that development of apparatus for correct measurement of bioluminescence in situ is quite a complicated problem. Depending on the intended purpose of the particular measurements, instrument requirements can vary substantially. Therefore, a set of instruments has to be used to obtain a sufficiently detailed study of bioluminescence.

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ON THE SENSITIVITY OF PHOTOMETERS FOR RECORDING BIOLUMINESCENCE /102

Theoretically, two systems of units can be used in the photometry of bioluminescence: light and energy (radiative).

A system of luminous particles is a system of effective values (Meshkov, 1957) corresponding to the sensitivity of the human eye. Therefore, this system units is used in the case where the spectral sensitivity of the receiver approximates the sensitivity of the average eye (so-called visibility curve) and in all cases characterizes the received radiation only from the point of view of its visibility to the eye. If the spectral radiation density at the detector is known, we can calculate effective values of visual sensation, but we can say nothing about the spectral radiant emittance, i.e., about the quantities characterizing the process itself, on the basis of a known effective light value.

At present, exclusive instruments with photomultipliers are used to measure bioluminescence in the sea and the sensitivity curves of these differ from that for the human eye. For these reasons, it is necessary to express spectral measurements in energy units for a quantitative description of luminescence. However, in examining some integral effects of bioluminescence (for example, questions of bioluminescence visibility and the like), it is desirable to consider the visual characteristics of luminescence as well. This is not only because of the high sensitivity of the human eye (which is not inferior to that of the best photomultipliers), but also because of the phenomenal capabilities of the human eye as an analyzer to "recognize" bioluminescence, to separate the "required" signal against the background of other signals. In this regard, not a single instrument can compare with the eye. This fact is quite important when studying such a complex phenomenon as bioluminescence, which, as a rule, also has a very low energy level. Therefore, the human eye can scarcely be replaced by an instrument when investigating some of the integral effects of bioluminescence, at least at the present level of technology.

A distinction is made between integral and spectral sensitivity for photomultipliers (Soboleva, et al., 1965).

The integral sensitivity (S) of photomultipliers used in the visible part of the spectrum is usually expressed as the relationship of the total photocurrent to the value of incident luminous flux of undispersed light from an incandescent bulb in mode A (tungsten filament temperature at 2,854 K). The unit of measurement of integral sensitivity is the ampere per lumen (A/lm). 103

The spectral sensitivity S_λ is defined as the ratio of the photocurrent to the monochromatic radiant power at wavelength λ. Spectral sensitivity is measured in A/W. Sometimes the spectral sensitivity is characterized by the value of quantum yield. Relative spectral sensitivity is defined as $S'_\lambda = \frac{S_\lambda}{(S_\lambda)_{max}}$, where $(S_\lambda)_{max}$ is the spectral sensitivity at the maximum of the spectral sensitivity curve. The relative spectral sensitivity curve of the S-6 photocathode used in the FEU-19A, FEU-29, and FEU-64 (most

often used in bathyphotometers) is given in Figure 47.

The relationship between the spectral and integral sensitivities of a photomultiplier is established by the following relation:

$$S_{\text{int}} = \frac{(S_\lambda)_{\max} \int S_\lambda F_\lambda d\lambda}{683 \int F_\lambda V_\lambda d\lambda} \qquad (1)$$

where F_λ is the spectral distribution of radiant power of the source (in absolute or relative units); V_λ is the curve of relative luminous efficiency (fig. 48). ("International Lighting Vocabulary", 1963); 683 (lm/V) is the maximum value of the spectral sensitivity of the average eye (corresponds to a wavelength of 554 mμ); λ_0 is the long-wave boundary of the spectral sensitivity curve of the photocathode. In this formula, it is useful to separate the factor (so-called conversion coefficient):

$$k = \frac{683 \int F_\lambda V_\lambda d\lambda}{\int S_\lambda F_\lambda d\lambda} \qquad (2)$$

which characterizes the detecting efficiency of the eye for radiation of a given spectral distribution F_λ compared with a given photodetector. For the S-6 photodetector without light filter when using standard source A, the coefficient of conversion is about 1,400 lm/W.

Thus, the relationship between the integral sensitivity of the photomultiplier and the spectral sensitivity (absolute) at the maximum of the spectral sensitivity curve of the photodetector is determined by the relationship:

$$(S_\lambda)_{\max} = k S_{\text{int}}. \qquad (3)$$

Usually, the values of the integral sensitivity (at several supply voltages) and the relative spectral sensitivity (determined by the type of photocathode) are entered in the photomultiplier data sheet. /104

The effective integral sensitivity (in A/W) with respect to the radiation with spectral emittance $F\lambda$ (absolute or relative) in the λ_1, λ_2 wavelength interval is:

$$S_{ef} = (S_\lambda)_{max} \cdot \frac{\int_{\lambda_1}^{\lambda_2} S'_\lambda F_\lambda d_\lambda}{\int_{\lambda_1}^{\lambda_2} F_\lambda d_\lambda} \qquad (4)$$

Actually, however, the sensitivity of photometers may substantially differ from that stated in the photomultiplier data sheet. This can be caused by a change in photomultiplier sensitivity during use, by using supply voltage different from that specified in the data sheet, by light filters, by absorption in the optics and by other factors. Consequently, there is a photodetectors used to record bioluminescence need for the sensitivity to be periodically calibrated. The calibration methods have to take into account the specific requirements of marine field work and the special features of the light-sensing cell in the bathyphotometer. These features are:

1. The high sensitivity of the photomultiplier and the relatively low dynamic range of the recording apparatus compel the use of standard light sources of low intensity, approximating that of the recorded signals.

2. The great instability of photomultipler sensitivity, the very great dependence of the sensitivity on power voltage. To reduce errors related to sensitivity changes, methods must be used that permit rather frequent calibration of instrument sensitivity (ideally) right at the time of the measurements. /105

3. Calibration has to be carried out under sea conditions--taking into account ship motion, sharp fluctuations in temperature, humidity, etc. Because of limitations of time and laboratory space during expeditions, calibration should not occupy much time or space.

Therefore, under field conditions, it is impractical to calibrate bathyphotometers on a photometric bench using incandescent bulbs as secondary light standards.

It would be more efficient to use a set of luminophors of constant luminescence with different brightnesses and spectral characteristics as light standards for calibrating bathyphotometers. A hermetic container with a luminophor activated by some radioactive isotope (for example, C^{14}) would be such a standard. The luminance of such standards is determined solely by the quantum yield of the luminescence and for all practical purposes depends solely on the half-life period of the isotope, i.e., is constant to a very high degree of accuracy.

For constant-luminescence standards, the brightness (in the filaments) and color of luminescence are designated in the data sheet. Since the luminescence spectrum of the standard is quite wide, the relative spectral intensity of the radiation of the standard must also be known for any energy computations. It can be measured by means of any photometer having the requisite sensitivity. Figure 49 shows curves of the relative radiation intensity of two standards used during the 38th expedition of the VITYAZ', taken on the ISP-51 spectrograph with the FEP-1 accessory. Corrections have been introduced in the curves for variation in instrument dispersion and photomultiplier sensitivity over the spectrum.

If the luminous flux (F, in lm) incident on the photodetector from the standard is known, then the effective integral sensitivity S'_{ef} (in A/lm) for the given photodetector and standard with given spectral radiation distribution is:

$$S'_{ef} = \frac{I}{F} \tag{5}$$

where I is the current at the photomultiplier output (in amperes), and the absolute spectral sensitivity at the maximum of photodetector sensitivity is:

$$(S_\lambda)_{max} = K S'_{ef}. \tag{6}$$

The coefficient of conversion in this case has to be computed for the spectral distribution of the radiation of the standard and the relative spectral sensitivity of the photodetector (when using a light filter whose spectral transmittance has been taken into account). In particular, for the standards whose spectral characteristics are given in Figure 49, and photomultipliers with S-6 photocathodes, the respective coefficients conversion are: K_I = 187 lm/wt K_{II} = 870 lm/wt /106

When calibrating under field conditions, the standard, which generally has a diameter of 20mm, is set up right in front of the detector window. The distance between the standard and the photomultiplier photocathode can be 4-5cm. In this case, when computing the irradiance generated by the standard on the photocathode surface, the finite dimensions of both standard and photocathode have to be considered.

The luminous flux (F) incident on the photodetector and the brightness (B) of the source in this case are related (in the transfer of radiation from a disc to a coaxial disc) by the equation (Sapozhnikov,1967):

$$F = \frac{\pi}{2} B \left[r_1^2 + r_2^2 + h^2 - \sqrt{(r_1^2 + r_2^2 + h^2)^2 - 4 r_1^2 r_2^2} \right]. \tag{7}$$

where $2r_1$ is the diameter of the standard, $2r_2$ is the diameter of the photocathode, and h is the distance between the photocathode and the standard. Hence, the mean irradiance of the photocathode is*

$$E = \frac{\pi B}{2\pi r_2^2}\left[r_1^2 + r_2^2 + h^2 - \sqrt{(r_1^2 + r_2^2 + h^2)^2 - 4r_1^2 r_2^2}\right]. \quad (8)$$

*Introduction of the concept of mean photocathode irradiance is only justified on condition that the light characteristic of the detector (output signal as a function of irradiance) is linear. For photomultipliers, this condition is well fulfilled in the absence of circuit overload.

Thus, to calibrate a photometer using constant-luminescence standards, the following computations have to be carried out: (a) the coefficient of conversion for the given standard and photodetector (if light filters are used, taking their transmission into account) has to be computed using formula (2); (b) the radiant flux incident on the photodetector from the standard, using formula (7). After this, formula (5) may be used to determine the effective integral sensitivity (in A/lm) for radiation similar in composition to that of the standard, and formula (6), for the absolute spectral sensitivity at the maximum sensitivity of the photodetector. This last quantity (together with the relative spectral distribution of sensitivity) characterized the photometer sensitivity without reference to the recorded radiation. When a rather wide-band photodetector is used, the effective integral sensitivity must be computed using formula (4). For computations with this formula, the form of the function of spectral density F_λ of the recorded radiation in the receiver pass band must either be known or assumed.

To increase the sensitivity of photometers used to record bioluminescence, light filters are usually not used and the entire radiant flux incident on the photodetector is recorded. In this case, an error associated with the development of the spectral composition of the standard radiation and the studied radiation is introduced in the measurement results. Very few measurements are presently being made of the spectral distribution of bioluminescence radiation in the sea. Figure 50 shows the mean spectral distribution of bioluminescence in the Red Sea (Karabashev, in press). These measurements were conducted with a rather crude spectral instrument. Therefore, only quite approximate estimates can be made of the error due to the nonmonochromatic nature of the luminescence of marine organisms. Computations of this error can be made on the basis of formula (4) following the relationship:

/107

$$\delta(S_{\max}) = \frac{S'_{\max} - S''_{\max}}{S'_{\max}} \quad (9)$$

where $S_{эф}^{эт}$ is the effective integral sensitivity (in A/W) for radiation with the standard spectral distribution, $S_{эф}^{изм}$ is the effective integral sensitivity (in A/W) for recorded radiation. Using standard II (fig.49), the error in the determination of sensitivity does not exceed 11%. In the case of calibration using standard I, the measured value will be about 80% higher.

The total sensitivity of the entire installation (S_{Σ}) is obviously equal to the product of the sensitivities of the photomultiplier and recording channel. For example, in direct recording of the photocurrent from the photomultiplier to the loop oscillograph.

$$S_{\Sigma} = S_{ФЭУ} \cdot S_{ш.о.} \qquad (10)$$

At the usual loop-oscillograph sensitivity (for example, H - 700 with a low-frequency loop) of 0.5 μA/mm, (with 2-mm oscillograph beam deviation), it is possible to record the luminescence of marine organisms creating a radiant flux up to $2 \cdot 10^{-6}$ μW on the photodetector cathode (FEU-29 with S-1000 A/lm). To convert to irradiance values, the radiant flux is divided by the photocathode area. The minimum irradiance that can be recorded is $3 \cdot 10^{-7}$ μW/cm^2. /108

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METHOD OF COMPARATIVE ANALYSIS OF THE BATHYPHOTOGRAMS OBTAINED WHEN LOWERING AND RAISING THE BATHYPHOTOMETER (SOUNDING METHOD) /115

An effective instrumental method of separating the astronomical component is the method of comparative analysis of the bathyphotograms obtained when lowering and raising the bathyphotometer at a constant rate (sounding method), first used by Uy. A. Rudyakov (1967b) in work involving measurement of bioluminescence in situ in the Red Sea. As the author notes, the luminescence of organisms is chiefly excited in the body of water disturbed by the movement of the instrument and not because of impacts against their bodies. In front of the object moving in the water the disturbed zone is small, while behind it vortices are generated. Therefore, intense bioluminescence is only recorded when raising the instrument (if the window is oriented downwards), and only occasional flashes are observed in lowering. The nature of the signal changes with depth during instrument descent corresponds to the nature of the change in the astronomical component (curve nearly exponential). During operations in a region of intense bioluminescence (Red Sea), signal amplitudes during instrument ascent and descent differ by a factor of 5-10, even when measurements are at a depth of 0-10m. Therefore, it is possible to assume with reasonable accuracy that during instrument descent only the astronomical component is recorded. Having data available on the illuminance on the photodetector recorded during instrument ascent and descent, it is not difficult to calculate the amount of biogenic light in the total illuminance in the horizon.

An interesting possibility may be noted of increasing bioluminescence recording efficiency in comparison with the astronomical component when "sounding" with the instrument window facing downwards. The body of the brightness of the astronomical component in sea water in the lower hemisphere has a minimum at about 0° and increases with increasing angle to $\pi/2$, while contrariwise the bioluminescence when the instrument is being raised is chiefly concentrated along the axis of instrument motion. Therefore, a decrease in the angle of view of an instrument with a plane photodetector results in a disproportionate attenuation of the radiant flux recorded by the instrument from the astronomical and bioluminescent components. Estimates show that when the angle of view of the instrument is decreased from π to about $\pi/2$, the ratio of bioluminescent light to the astronomical component almost doubles, while the total recorded signal when the angle is decreased to $\pi/2$ due to limitation of radiant flux incident on the photodetector decreases insignificantly. /116

Computational Method of Separating the Astronomical Component-- We have used a computational method based on the following assumptions:

1. There are no grounds for assuming that the attenuation of natural light characterized by the index of vertical attenuation in the case of almost identical source geometry will depend on the general level of surface illumination. Numerous observations have shown that the index of vertical attenuation (Kozlyaninov, 1961) depends to a greater degree on the optical properties of the water than on the function of the source (in any case, at the depths of interest to us).

2. In the surface layers of the water, the astronomical component is usually controlling. According to data obtained by Clarke (Clarke and Denton, 1962), on a moonlit night, the brightness of the astronomical component is two orders greater than the brightness of bioluminescent flashes. On moonless nights, brightnesses are comparable, but owing to their scarcity the role of bright flashes is not great, while the mean brightness of the flashes is an order less. In addition, the maximum level of bioluminescence is, as a rule, observed not on the surface, but usually at a depth of 50-70m. Therefore, the assumed predominate role of the astronomical component at the surface in some cases remains valid (though with less accuracy) on bright moonless nights as well.

The values of the index of vertical attenuation α in the region of interest to us (from the point of view of bioluminescence investigations) can be obtained directly, after first having measured the curve of irradiance with depth with an appropriate instrument during the daylight hours, or by using measurements made earlier in the required region. On the basis of a known α value from a surface point with measured irradiance, the curve of underwater irradiance due to the

astronomical component to the depths of interest, can be constructed. A graph showing the dependence of the constant background of bioluminescence on depth is obtained as the difference between the curve of measured total luminescence and the computed curve of underwater irradiance due to astronomical factors.

As an example, let us examine records made on one of the Pacific Ocean stations (fig. 52). Along the axis of abscissas, total illumination at each horizon is plotted; along the ordinate axis, the depths. Curve (1) has a sharply defined maximum at a depth of 34m. With decreased or increased depth from 34m, the luminescence intensity decreases smoothly. The change of astronomical illumination with depth (3) is shwon. Points for the construction of curve (3) were determined by the formula: /117

$$E_z = E_{zo} \cdot 10^{-a(z-zo)} \qquad (27)$$

where E_z is the illumination value at depth Z; E_{zo} is the illumination value at depth Z_o; a is the index of vertical attenuation (decimal); Z is the depth for which illumination is being determined; Z_o is the depth for which the illumination value is known and from which the curve is plotted. The values Z_o = 1.5m, and Z = 86m, may be assumed. In the records made at depths of 1.5 and 86m, pulses are quite rare; Consequently, the constant component of the record can be taken as the record of the value of astronomical illumination at these depths (E_z; E_{zo}). Transforming formula (27) in the form:

$$a = \frac{\lg E_{zo} - \lg E_z}{z - z_o} \qquad (28)$$

and inserting known values in it, we get $a = 0.024m^{-1}$.

Using the value of a obtained, we determine the illumination for several depths from 1.5 to 86m. On the basis of the illumination values obtained, the curve of variation of astronomical illumination (3) is constructed.

Comparison of the three curves in Figure 52 shows that the maxima of curves (1) and (2) coincide. Curve (1) falls off smoothly above and below the depth of the maximum. Curve (2) also falls off smoothly below the depth of the maximum, while above the depth of the maximum it falls off to a depth of approximately 20m, and then rises again. Curve (3) descends by an exponential law from a depth of 1.5m. The amplitude /118 difference between curves (2) and (3) shows graphically that the constant component recorded by the bathyphotometer greatly exceeds the value of astronomical illumination and represents the sum of a number of values. Bacterial luminescence and the constant bioluminescence generated by the multitude of organisms outside the limits of the volume examined by the bathyphotometer are among them. At the depth of maximum luminescence, the predominant value in the total constant

component is contributed by the constant bioluminescence and confluent flashes of long duration. This explains the coincidence of the maxima in curves (1) and (2) and the general rise in curve (2) in the region of maximum luminescence. The rise of curve (2) above 20m is explained by the predominance of astronomical illumination which increases exponentially as the surface is approached in the total constant component.

METHODS OF MEASURING BIOLUMINESCENCE SIGNALS

Here it is expedient to examine some questions of method that arise when bioluminescence is measured in two ways: by means of an instrument located at a specific horizon and by continuous sounding. The methodology of measuring bioluminescence in the sea by means of apparatus dark-chamber, apparatus to record spontaneous bioluminescence, etc., is not examined here, since it is primarily influenced by instrument design and was examined earlier.

Measurements with Instruments with an Uncontrolled Excitation Level ("Standard" Bathyphotometers). We have already noted that an instrument located at a certain horizon in the sea's depths unavoidably disturbs the medium, thereby creating an increased bioluminescence level where it is. Therefore, the recorded signal to a considerable degree is determined by the presence of the instrument itself at the measurement point and is very closely related to its design features--the hydrodynamic characteristics that determine the level of excitation introduced, and by the arrangement of the optical part. The effect of the design of the instrument's optical part was examined above. The instrument itself introduces an error in the amplitude distribution of bioluminescent signals.

On the basis of Lyapunov's well-known theorem*, it may be assumed

*If the values of a random quantity are small compared with their sum (which is completely admissible in our case), then when the numbers of these values increase limitlessly, the distribution of their sum becomes approximately normal (Venttsel', 1962).

that the amplitudes of bioluminescence signals relative to the measurement point are distributed according to the normal law. Analysis of the statistical series constructed from measurement data obtained in the Atlantic Ocean in the nighttime confirms that the amplitude distribution differs clearly from the normal. Figure 53 shows the trend of the probability density curve constructed from data of a statistical series smoothed with the aid of a type I_j Pearson curve.

/119

Such deviation from the normal distribution cannot be explained as random. The construction of probability-density curves from statistical series compiled from measurement data obtained by the same type instrument at different points in the oceans of the world shows that they can all be smoothed by means of the same curve.

Analysis of the probability-density distribution curve constructed from experimental data could indicate the following: The probability of a bathyphotometer recording large amplitude light pulses is slight. Actually:

1. If, for ease of consideration, it is assumed that all bioluminescents produce light flashes of the same amplitude, then evidently the amplitude of the light flashes recorded by the bathyphotometer will be inversely proportional to the square of the distance to the bioluminescent themselves (it is assumed that absorption by the medium is negligible). From this the conclusion suggests itself that the bathyphotometer does not select bioluminescent signals located at different distances from the light detector with equal probability. Thus, the probability of recording high-amplitude light pulses is significantly lower than the probability of recording low amplitudes. Actually, considering the solid angle of view of the bathyphotometer, it may be noted that when there is an equiprobable distribution of bioluminescents at the point of measurement, the number of bioluminescents in the field of view of the bathyphotometer increases with distance away from the light-detector cathode.

2. When, because of ship motion, the bathyphotometer moves, a hydrodynamic wave (turbulence) is generated in front of its window. The excitation effect of the wave on the bioluminescents decreases /120
with distance from the window. On the basis of shipboard laboratory observations of individual organisms, it can be assumed that bioluminescents try to leave the zone of maximum effect of the exciting force. Considering the considerable latent period between the effect of the excitation force and the organism's response to it, it can be said that the bathyphotometer (or more exactly, its movement) introduces some distortion in the spatial distribution of bioluminescents at the point of measurement. Moreover, the existence may be postulated of bioluminescents capable of detecting the presence of a zone of alien turbulence and which try to avoid contact with it. In this case, the unequal probability of selection of bioluminescent flashes by the bathyphotometer increases. Obviously, the predominance of weak flashes in the surface layer and some increase with depth of the proportion of bright flashes are related to these two factors.

When analyzing bathyphotograms recorded when the instrument is at a specific depth, it is convenient to take the value of mathematical expectation of the luminous emittance for the value characterizing the bioluminescent luminous emittance at the point of measurement. The mathematical expectation of a random function is some average function around which all possible realizations are grouped. For a random function X(t), the mathematical expectation can be calculated by the formula:

$$m_x(t) = \int_{-\infty}^{\infty} x\omega(x,t)\,dx, \tag{29}$$

where $\omega(x,t)$ is the probability density.

Considering the relatively short time of a bioluminescence measurement, the distribution of bioluminescents in the horizon can be considered stationary. For stationary random processes, the mathematical expectation does not depend on time and is a constant. If, however, the process is not only stationary but possesses an ergodic property* as well (which is entirely admissible in

*The distinguishing characteristic of an ergodic process consists in that the probable characteristics can be obtained with a probability as close to unity as wanted as a result of a certain time averaging operation of one realization of sufficiently long (practically infinite) duration.

measurements of bioluminescent signals), then in such a process the average over the aggregate is equal to the time average determined from one realization $m_x(t) = \overline{x(t)}$. In this case, the average can be found with the formula:

$$\overline{x(t)} = \lim_{T\to\infty} \frac{1}{2T} \int_{-T}^{T} x(t)\,dt. \tag{30}$$

This equation assumes that the realization x(t) has infinite duration. However, as we have already said, in reality the measurements are made within a limited time period. For practical purposes, the following formula can be used to compute the time average (mathematical expectation): [12]

$$x(t) = \frac{1}{T} \int_{0}^{T} x(t)\,dt. \tag{31}$$

Let us note that in this case we obtain, not the true average value, but an estimate of it. This estimate will be closer, in the probability sense, to the mathematical expectation, the longer the measurement interval (measurement time can be considered sufficiently great if it is rather large compared to the maximum interval of correlation τ_{mc}*). Measurement of the average value of an ergodic

*The maximum correlation interval τ_{mc} is the interval beyond whose limits the correlation is negligibly small, i.e., the value of the normalized correlation function at $\tau = \tau_{mc}$ is smaller than any before given value of ε.

random process by time averaging one realization of sufficiently long duration can be carried out by relatively simple instrumental methods. Among possible "averagers" are: an RC circuit; an integrating circuit constructed on the basis of a dc amplifier with deep negative feedback; a moving-coil milliameter, etc.

The main source of error in computing the average value of bioluminescence is the finite duration of the measurement interval. The estimate dispersion can serve as an index of the accuracy of determination of this value:

(32)

$$D[m_x] = M\{[M_T(x)]^2\} - m_x^2.$$

For a stationary ergodic random process x(t), the estimate dispersion can be determined by the formula:

(33)

$$D[m_x] = \frac{2}{T}\int_0^T \left(1 - \frac{\tau}{T}\right) R_x(\tau)\,d\tau.$$

where $R_x(\tau) = M[(x(t) - m_x)(x(t+\tau) - m_x)]$

is the correlation function of the random process.

Taking into account that the integration time $T \gg \tau_{max}$, we will get:

$$D[m_x] \approx \frac{2}{T}\int_0^T R_x(\tau)\,d\tau. \tag{34}$$

It is known from the theory of mathematical statistics that [122]

$$R_x(\tau) = \rho(\tau)\sigma^2, \tag{35}$$

where $\rho_x(\tau)$ is the normalized correlation function.

In this case:

$$D[m_x] \approx \frac{2}{T}\int_0^T \rho_x(\tau)\,d\tau. \tag{36}$$

Let us introduce the concept of correlation interval τ_k:

$$\tau_k = \int_0^T \rho_x(\tau)\,d\tau \tag{37}$$

The quantity τ_k gives an idea of the time intervals at which correlation takes place between values of the random process.

Then:

$$D[m_x] \approx \frac{2}{T}\tau_k. \tag{38}$$

Taking into account $D[m_x] = \sigma_m^2$ (the square of the standard deviation), we get:

$$T = 2\frac{\sigma_x^2}{\sigma_m^2}\tau_k. \tag{39}$$

To solve most problems of bioluminescence, an accuracy of 10% is adequate, i.e., the standard deviation must be 10% of the standard deviation of the analyzed process itself ($\sigma_m = 1/10\,\sigma_x$). Then:

$$T \simeq 200\tau_k.$$

Computer computations show that when measurements are made at the 0-75m horizons (in the zone of the most fully developed "confluent" flashes) the value of τ_k can be taken as 0.8-1 sec. In conducting measurements at horizons deeper than 100m, the value of τ_k can be taken as 0.2-0.5 sec. Thus, the averaging interval T varies with the depth of the investigated horizon. For instrumental simplification of the "averagers" it is obviously sensible to use the maximum averaging interval regardless of the horizon of measurements. The averaging accuracy will then increase with increasing depth of the measurement horizon (of course, the measurement time at the horizon must ensure statistical reliability of the information obtained).

Sounding Method--One of the most promising methods of studying plankton luminescence in situ is the sounding method with a constant rate of bathyphotometer movement. The advantage of this method is determined by the following circumstances: /123

1. A bathyphotometer at a certain depth records luminescence, that to a considerable degree is caused by the presence of the instrument as was shown above. The level of excitation in this case will not remain constant from station to station or even from one horizon to another. When, however, the instrument moves at a constant and adequately high speed, the level of excitation generated by it is more definite, and the lack of data comparability which is possible during prolonged instrument exposure at a horizon is to a considerable extent eliminated.

2. When sounding narrow layers with high luminescence, the location of luminescence maxima is clearly delineated. When operating at fixed depths this requires too great a time expenditure and is not always possible.

3. The time for carrying out one series of measurements is sharply reduced (at least by a factor of 10). This permits more accurate recording of the time of bioluminescence measurements and when needed the carrying out repeated soundings.

4. The problem of separating the astronomical and bioluminescent components of the recorded signal is quite simple.

5. When using the sounding method, correlating plankton numbers with bioluminescence intensity is justified (Rudyakov, 1967b; Rudyakov, Voronina, 1967).

The selection of sounding speed is important in using this method, since it determines the disturbance level caused by the apparatus. At low speeds, because all bioluminescents are not excited, dependence of signal on speed can be expected. When the speed increases, an excitation level is reached at which this dependence disappears. Obviously, it is desirable to use the optimum speed.

When analyzing data obtained by sounding, the nature of the bioluminescence has to be considered, inasmuch as the duration of luminescence of one organism is comparable to the characteristic time the instrument is located at the measurement point. The [illegible] the instrument is at the measurement point can be characterized by the relationship r_o/v, where r_o is the minimum distance from which the flash can be detected (it is determined chiefly by the thickness of the instrument window and the character of the flow of water around the instrument as it moves), and v is the speed of

instrument movement. The necessary correction can be conveniently computed for a triangular pulse with vertical leading edge and a decay determined by the law $(1 - t/\tau)$, where τ is the pulse duration along the base. This bioluminescence pulse form is quite close to the actual. Solution of the problem for pulses with an exponential decay involves very laborious computations, but the result is quantitatively very close. /124

If maximum flash brightness is B, and the flash occurs at distance r_0 from the photodetector, then in the case where the instrument and source are fixed relative to each other, the detector records energy (per unit area):

$$U_0 = \frac{1}{2}\frac{B\tau}{r_0^2}. \tag{40}$$

If the instrument moves away from the source, it is easy to see that the recorded energy will be:

$$U = \frac{B}{r_0^2}\int_0^{\tau}\left(1 - \frac{t}{\tau}\right)\frac{dt}{1 + at} \quad \left[\frac{B}{r_0^2}\left(\frac{1}{a} - \frac{\ln(1 + a\tau)}{a^2\tau}\right)\right] \tag{41}$$

Here $a = V/r_0$, where V is the sounding speed, r_0 is the minimum distance between the photodetector and the organism at which a flash begins. Thus, the relationship of the energy recorded when the instrument is moving to the energy recorded by a fixed detector (distortion of recorded energy) is:

$$\delta(a, \tau) = \frac{U}{U_0} = \frac{2}{a^2\tau}\left[\frac{a\tau}{1} - \frac{\ln(1 + a\tau)}{a^2\tau}\right]. \tag{42}$$

Radiation from bioluminescent flashes of different duration and maximum brightness is incident on the photodetector. If the distribution of the total energy recorded by the instrument as a function of flash duration is given (such a distribution can be obtained, for example, when the instrument is measuring at a fixed horizon), then the total distortion of the recorded energy is:

$$\delta(a) = \sum p(\tau_i)\,\delta(a, \tau_i) \tag{43}$$

where $p(\tau_i)$ is the fraction (statistical weight) of bioluminescent energy concentration in flashes having a duration of $\tau_i \pm h/2$; (h is the summation step). In particular, for the distribution of total energy according to the flash duration characteristic of the central Pacific Ocean, and $r_0 = 0.1$m (which is realistic for the instruments employed), the following correction values for the sounding velocity /125

are obtained by which it is necessary to multiply the measured energy values.

Speed m/sec.	δ/a	Speed m/sec.	δ/a
0.?	1.?5	1.0	2.00
0.5	1.59	1.5	2.?8
0.7	1.7?	2.0	2.78

As is evident from the data presented, the correction is substantial. Failure to apply the correction can lead to an error in determining the sounding speed that ensures the requisite excitation level.

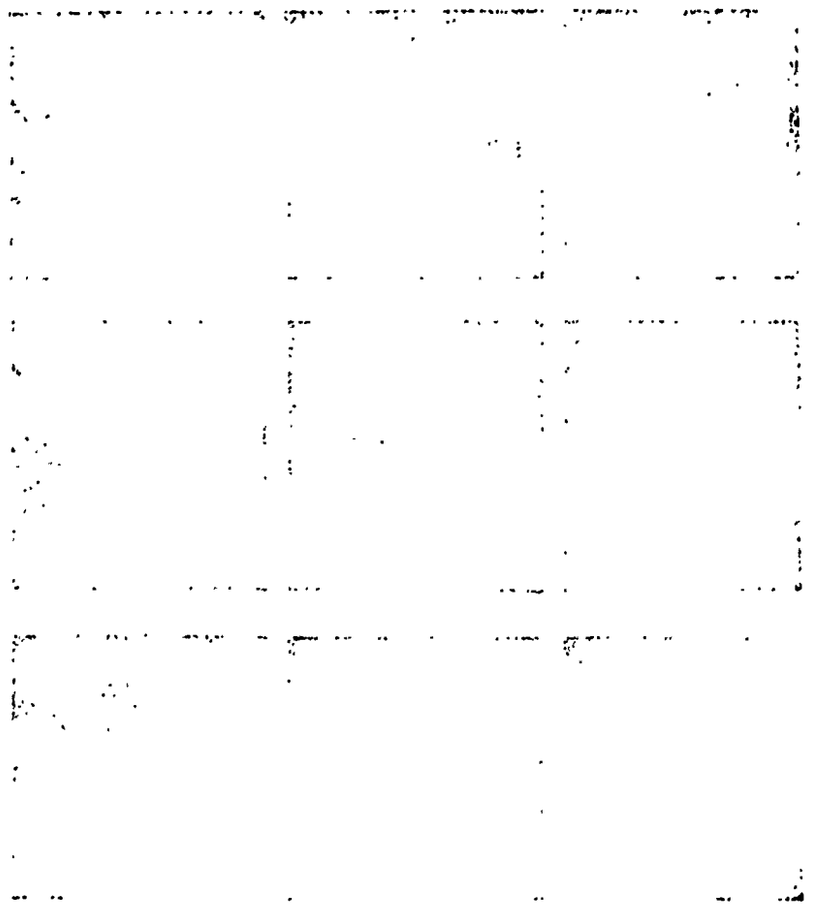

Fig. 1. Luminescence of organelles of Noctiluca miliaris (Eckert, Reynolds, 1967). Photographs obtained with image converter: a_1, b_1, and v_1--low amplification level (dark-field photograph), object to left of cell is tip of pipette; a_2, b_2, and v_2--luminescence of same three cells, high amplification; a_3, b_3, and v_3--luminescence after several minutes.

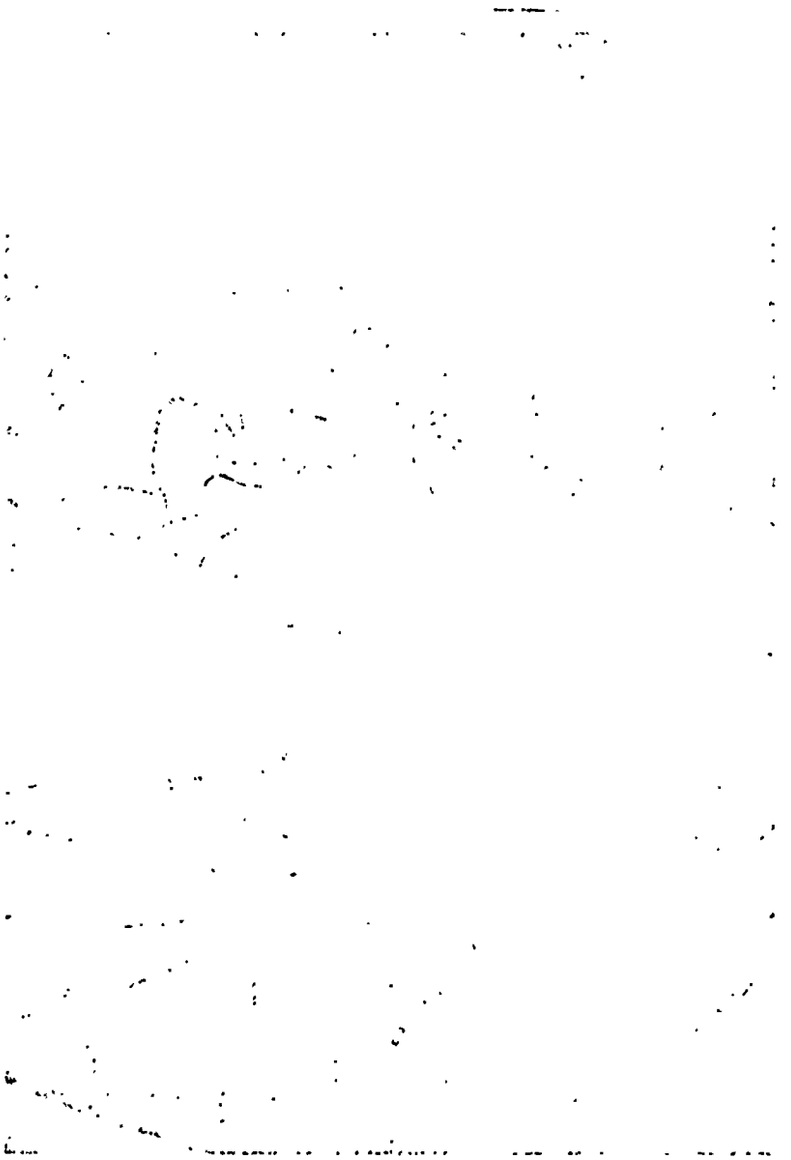

Fig. 2. Electron microscopic photographs of the cytoplasm of Gonyaulax polyedra (Sweeney, Bouck, 1966): S = scintillons

Fig. 3. Jellyfish Aequorea aequorea: a--arrangement of photogenic tissue; b--side view; v--from oral side (Johnson, Snook, 1927; from Harvey, 1952)

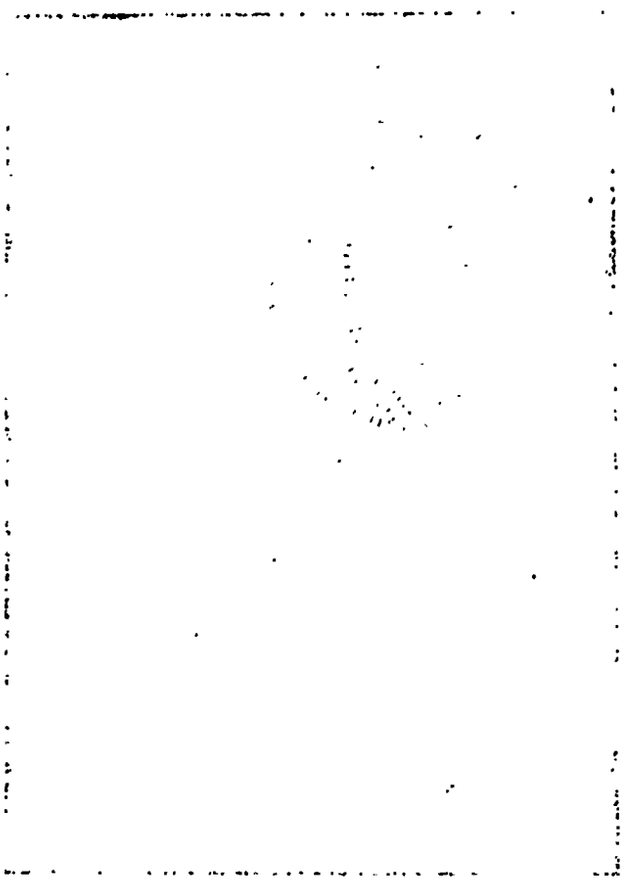

Fig. 4. The ctenophoran Pleurobrachia pileus at the moment of luminescence (Dahlgren, 1916).

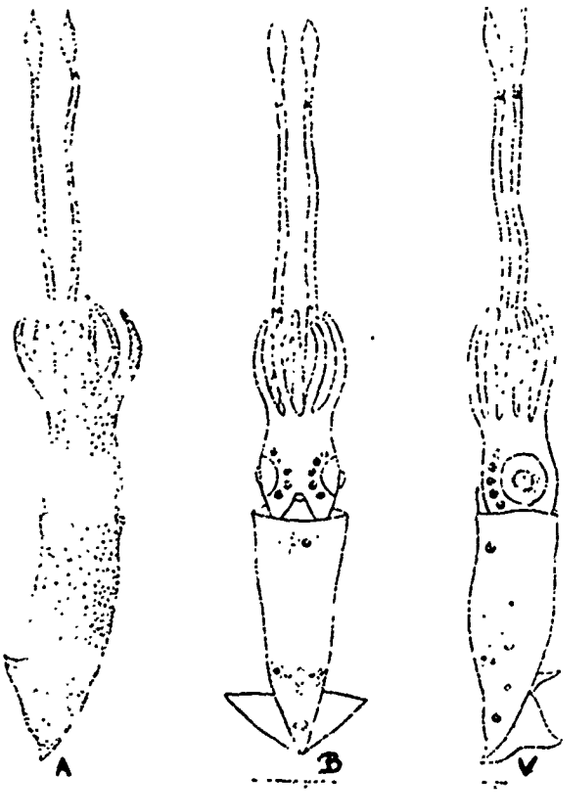

Fig. 5. Luminous squid Lycoteuthis diadema: Side view (a, v) and ventral view (b) (Chun, 1910; from Nicol, 1960a)

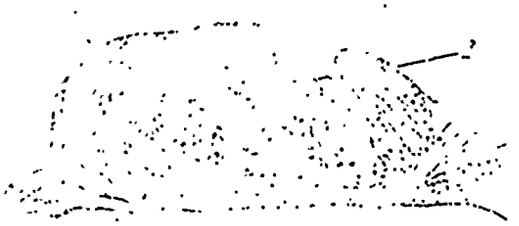

Fig. 6. Simple luminous organ of squid Liocranchia valdiviae (Dahlgren, 1916): 1--reflector; 2--luminous body

Fig. 7. Structure of complex photophore of squid Calliteuthis reversa: 1--reflector; 2--luminous body; 3--opaque pigmented layer; 4--chromatophore screen; 5--lens; 6--inner lens cords (Chun, 1910; from Nicol, 1960a)

Fig. 8. Luminescent mollusc Phyllirrhoe bucephala (Panceri, 1873; from Harvey, 1952)

Fig. 9. Mollusc Pholas dactylus (luminescent zones indicated in black) (Panceri, 1872; from Nicol, 1960a)

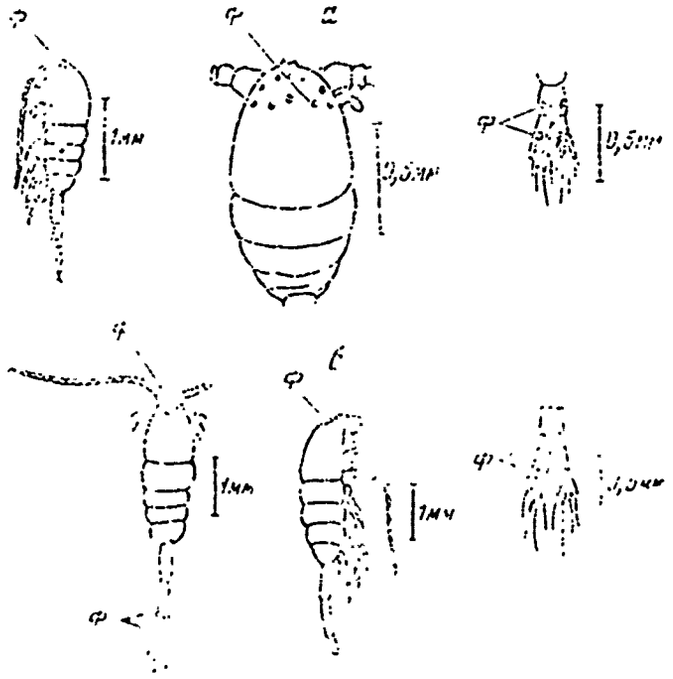

Fig. 10. Location of fluorescent luminous glands in Metridia lucens (a) and M. longa (b) (Clarke et al., 1962) Ф--fluorescent parts

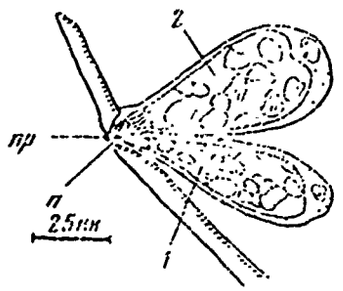

Fig. 11. Structure of luminous gland of head of Metridia longa. Transverse horizontal section: n--pore; np--secretion sample: 1--type I glands; 2--type II glands.

Fig. 12. Luminescent fish Argyropelecus hemigymnus. Photograph by Y. Haneda (McElroy, Seliger, 1962)

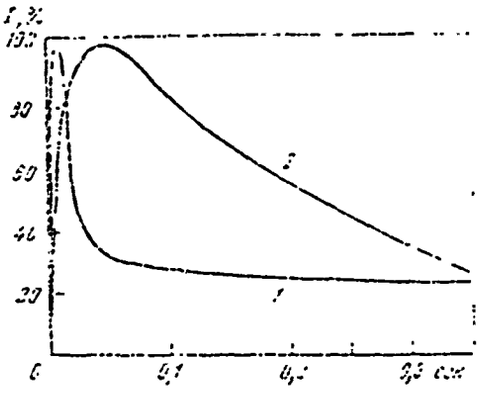

Fig. 13. Luminescence of Cypridina hilgendorfii in vitro: 1--O_2 is added after luciferin and luciferase are mixed; 2--reaction components before admixture of O_2 carrier (Chance, et al.,1940)

Fig. 14. Pyloric appendages and internal organs of Parapriacanthus ransonneti photographed in daylight (a) and in ultraviolet (b) (Haneda, Johnson, Shimomura, 1966)

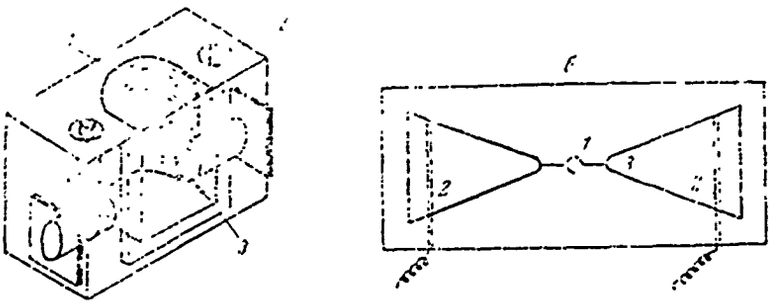

Fig. 15. Cuvette for laboratory investigation of luminescent flares of separate organisms: a--after David, Conover, 1961; 1--central working chamber; 2--carbon electrodes; 3--microfilter; b--after Nicol, 1958c: 1--central chamber; 2--silver electrode; 3--agar transition.

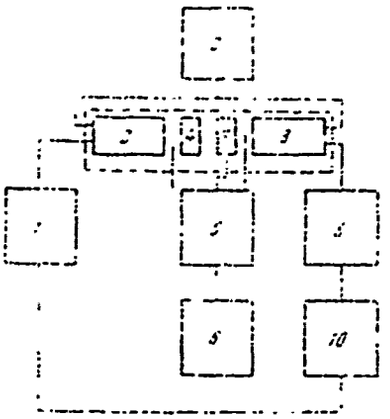

Fig. 16. Block diagram of laboratory setup for investigating luminescent flashes of individual organisms (Institute of Physics of the Siberian Division of the USSR Academy of Sciences). 1--d.c. amplifier; 2--photomultiplier; 3--power supply; 4--interchangeable light filter and reference-standard unit; 5-6--electric pulses generated; 7--cuvette with luminescents under study; 8--photomultiplier; 9--switch and power supply unit; 10--loop oscillograph.

Fig. 17. General view of laboratory apparatus (Institute of Physics of the Siberian Division of the USSR Academy of Sciences).

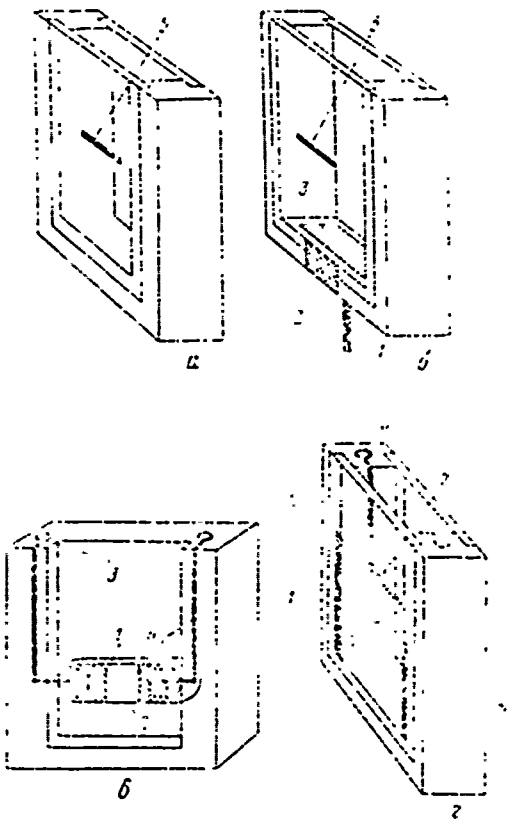

Fig. 18. Cuvettes for investigating flashes of individual organisms: a--cuvette for chemical stimulation; b--cuvette for mechanical stimulation; 1--flexible steel rod; 2--soft rubber diaphragm; 3--organic glass; v--type I cuvette for electrical stimulation: 1--agar bridges; 2--working space; 3--contacts to connect electrodes; 4--agar-silver electrodes; g--type II cuvette for electrical stimulation: 1--electrodes made of noncorrosive metal; 3,4--same as for "v"; for a, b, g: 5--line for checking water level in cuvette.

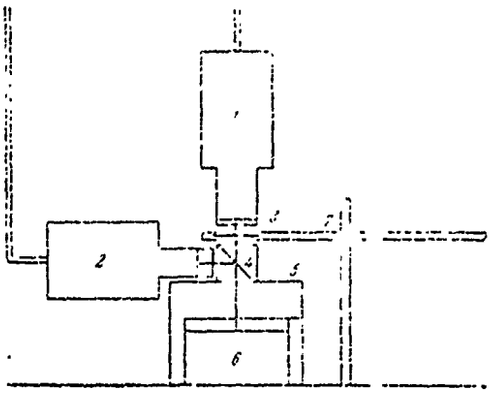

Fig. 19. Design diagram of laboratory equipment for studying fish luminescence: 1--vertical photomultiplier; 2--horizontal photomultiplier; 3--light filter; 4--semitransparent plate; 5--dark chamber; 6--vessel with sea water; 7--disc with light filters.

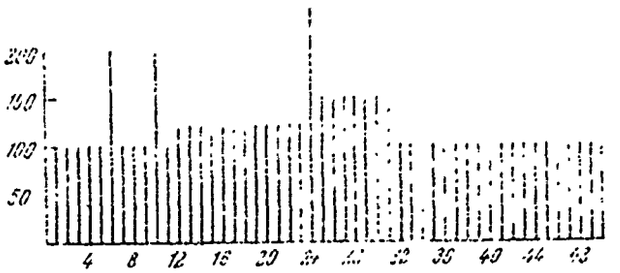

Fig. 20. Duration of Noctiluca miliaris flashes in the case of successive stimulations (0.? Hz; 4.4 ms; 1 mA/mm^2).

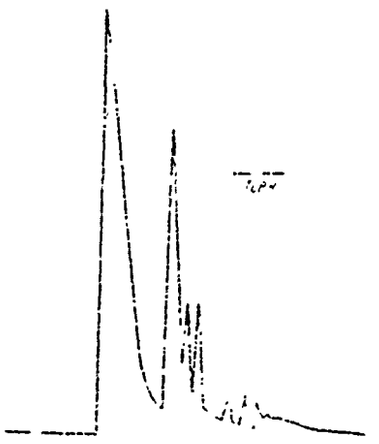

Fig. 21. Complex Noctiluca miliaris flash in the case of chemical stimulation.

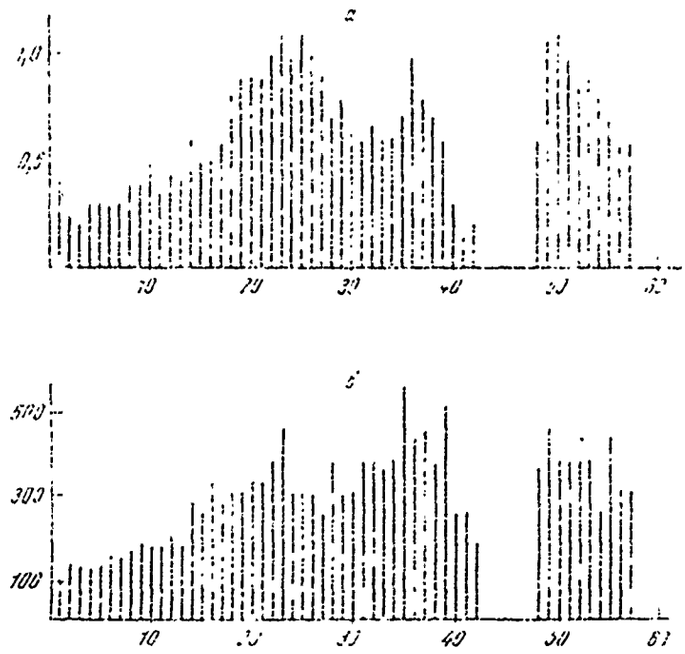

Fig. 22. Amplitude (a) and duration (b) of Pyrocystis pseudonoctiluca flashes under repeated stimulation.

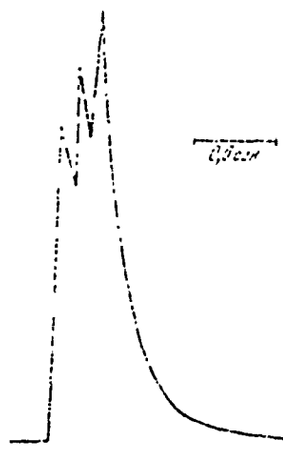

Fig. 23. Flash of Pleuromamma sp. under electrical stimulation.

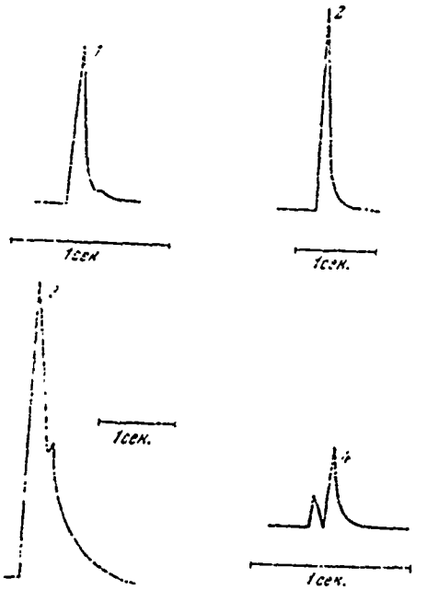

Fig. 24. Bioluminescent signals of some Copepoda (Greze et al., 1966): 1--Scolecithrix danae; 2--Candacia dactyla; 3--Lucicutia gemina; 4--Undinula vulgaris.

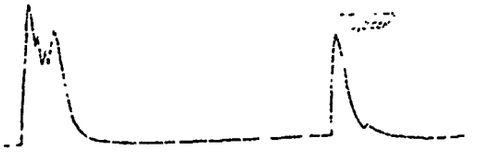

Fig. 25. Successive flashes of Oncaea sp. under electrical stimulation (1 Hz, 5 ms, 8 mA/cm^2).

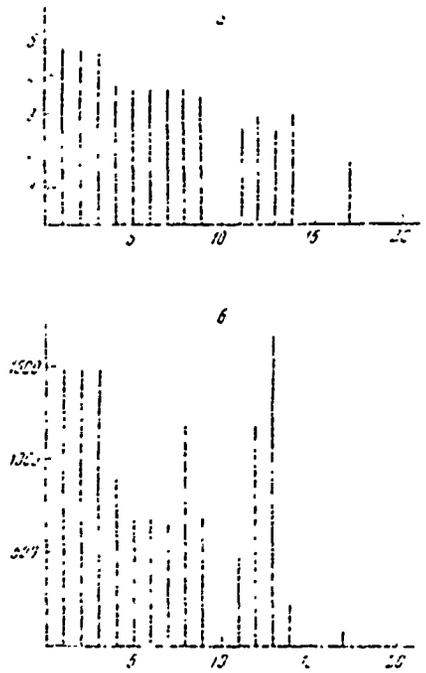

Fig. 26. Amplitude (a) and duration (b) of flashes of Metridia sp. under repeated stimulation (1 Hz, 10 ms, 7.5 mA).

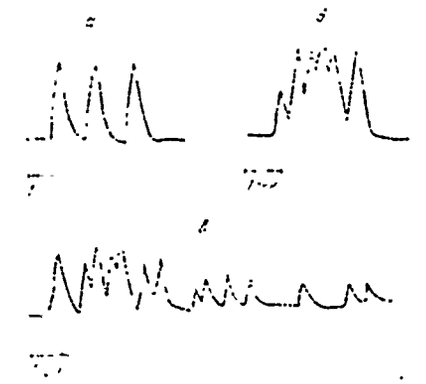

Fig. 27. Bioluminescent signal of Astronesthes niger under mechanical (a, b) and chemical (v) stimulation.

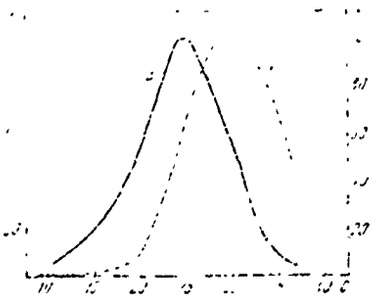

Fig. 31. Dependence of growth (1) and luminescence (2) of Photobacterium sp. (strain no. 5) on temperature. I--intensity of luminescence; D--optical density of culture.

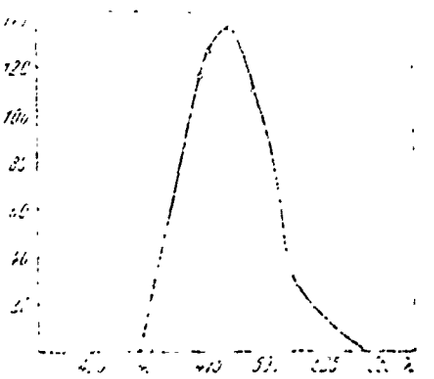

Fig. 32. Luminescence spectrum of Photobacterium fischeri (Strehler, Cormier, 1952).

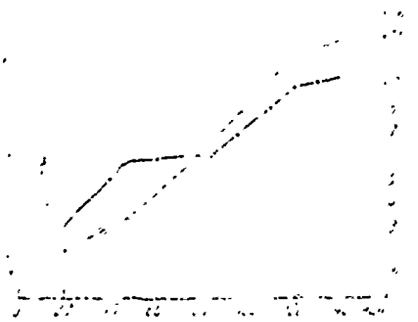

Fig. 33. Luminescence (1) and growth (2) of synchronized culture. N--culture concentration; I--bioluminescence intensity.

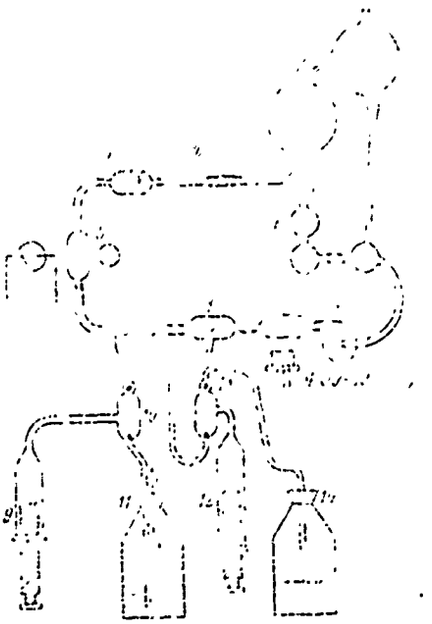

Fig. 34. Diagram of installation for continuous culture of luminous bacteria (Gitel'zon, Fish, Chumakova, 1967). 1--centrifugal pump; 2-3--flow-through luminescence and temperature recorder cuvette; 4--connection to inflow-outflow system; 5--flow-through optical density sensor cuvette; 6--oxygen sensor; 7--air input sector; 8--vortex gas and foam separator; 9-10--nutritive medium input micro pump; 11--vessel; 12-13--suspension output pump to vessel; 14--vessel.

Fig. 35. Luciferase crystals (a) and NAD-H_2-oxyreductase crystals (b) (Borkhsenius, et al. 1967).

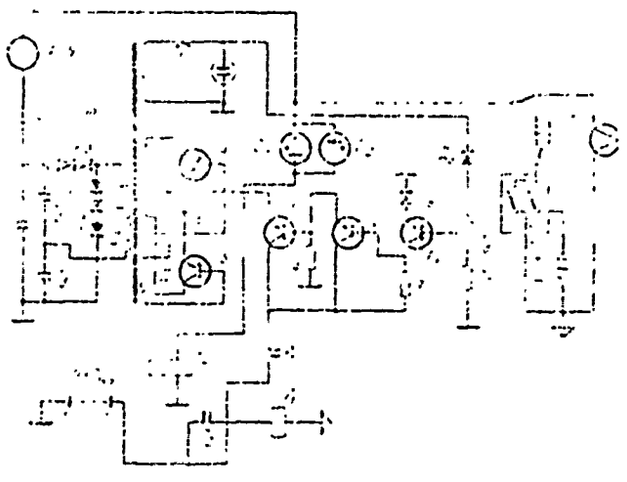

Fig. 40. Basic circuit of bathyphotometer (Institute of Physics of the Siberian Division of the USSR Academy of Sciences).

Fig. 41. Overall view of the submerged part of the bathyphotometer (Institute of Physics of the Siberian Division of the USSR Academy of Sciences).

Fig. 42. Portable bathyphotometer set (Institute of Physics of the Siberian Division of the USSR Academy of Sciences).

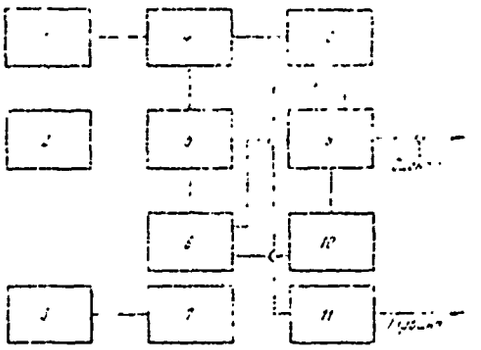

Fig. 43. Block diagram of the submerged part of the logarithmic bathyphotometer (Institute of Physics of the Siberian Division of the USSR Academy of Sciences). 1--remote switch-on unit; 2--power-supply unit; 3--automatic-calibration program device; 4--voltage stabilizer; 5--photomultiplier power-supply-voltage transformer; 6--photomultiplier; 7--mechanical gate; 8--voltage transformer of tube and semiconductor amplifier power-supply; 9--amplifier unit and photomultiplier sensitivity control circuits; 10--photomultiplier power-supply divider; 11--depth gage.

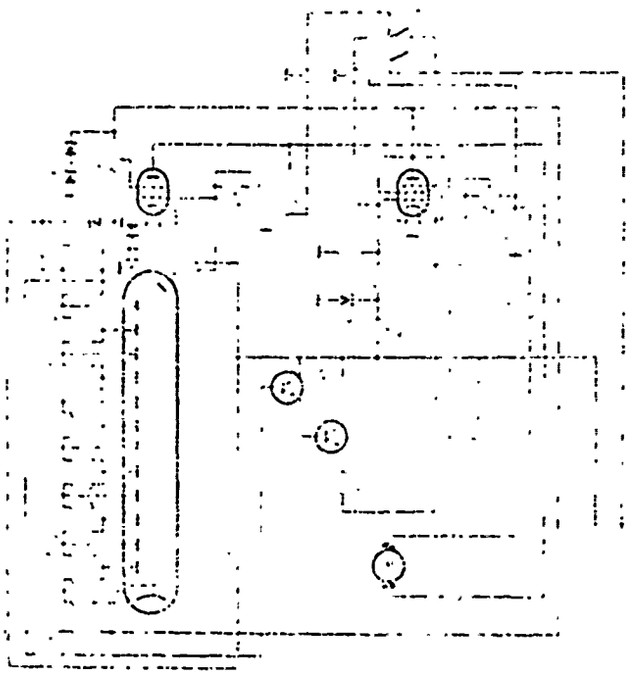

Fig. 44. Basic circuit of submerged part of logarithmic bathyphotometer (Institute of Physics of the Siberian Division of the USSR Academy of Sciences).

Fig. 45. Overall view of the submerged part of the logarithmic bathyphotometer.

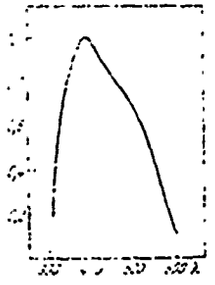

Fig. 47. Relative-spectral-sensitivity curve of type S-6 photocathode.

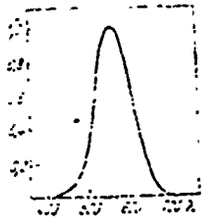

Fig. 48. Relative visibility curve for daytime vision.

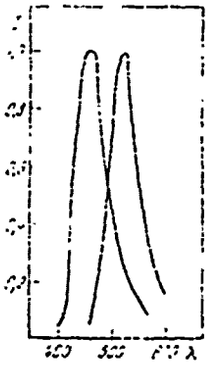

Fig. 49. Relative spectral intensity curves (in relative units) of two radiation of two standard luminophors.

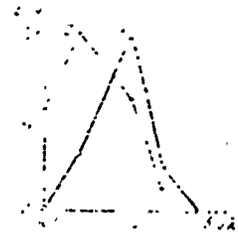

Fig. 50. Mean relative spectral distribution of the bioluminescence radiation in the Red Sea (1) and the relative spectral distribution of an ascending current of natural radiation (2) (Karabashev, in press).

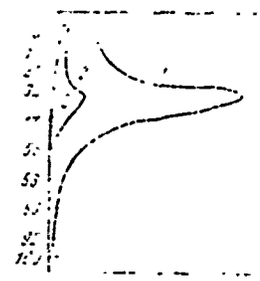

Fig. 52. Depth distribution of bioluminescent and astronomical components. 1--total recorded signal; 2--variation of constant component for horizons; 3--change of astronomical illumination with depth.

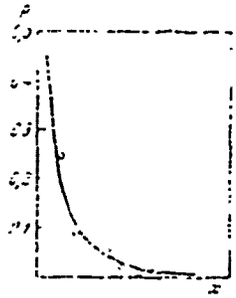

Fig. 53. Approximate trend of probability-density curve of bioluminescent flash amplitude distribution.